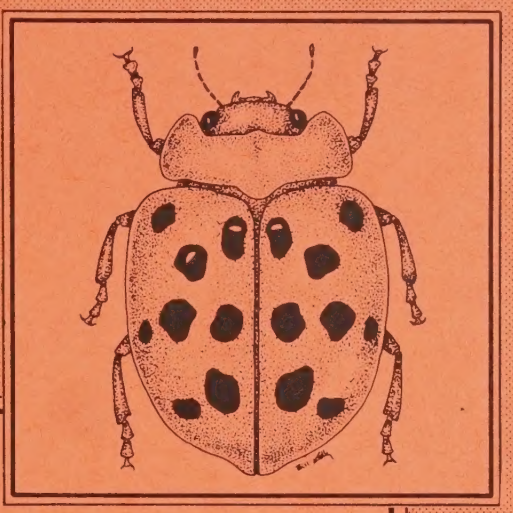

United States Department of Agriculture

Otis Methods Development Center

Progress Report

Animal and Plant Health Inspection Service

October 1, 1983 - September 30, 1984

Laboratory Report
Otis Methods Development Center
Animal and Plant Health Inspection Service
United States Department of Agriculture
Otis Air National Guard Base
Massachusetts
02542

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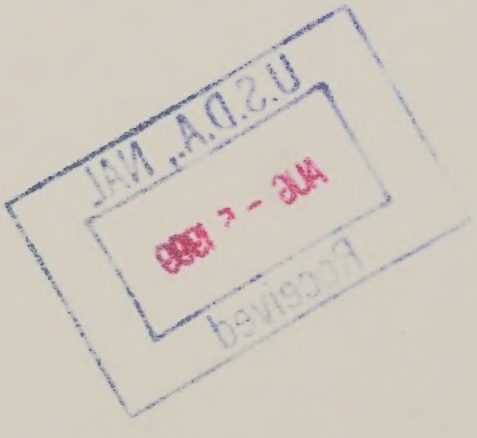

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Summary

The search for treatments useful in gypsy moth regulatory activities was continued, and a number of chemical insecticides and innocuous materials were identified as having ovicidal properties. The materials, norpine, creosote, soybean oil and isopropyl alcohol all devitalized gypsy moth eggs, regardless of the time of the year that the treatments were applied. These agents will be useful for treating egg masses on regulated items. Careful inspection is necessary, however, since the treatments are effective only when applied directly to the egg masses.

Numerous laboratory tests were conducted with a new strain of Bt developed by Norman DuBois, U.S. Forest Service. It is identified by Zoecon as SAN-415 and Abbott as ABG-6163. Generally, formulations of this strain were shown more effective than standard strains.

Feeding bioassays were conducted which showed that all Bt formulations strongly deter feeding when untreated foliage is offered as a choice. Phytotoxicity determinations were done with several Bt mixes: Poplar, birch, maple, catalpa, and blueberry were all highly sensitive, whereas ash, locust, peach and plum showed no evidence of phytotoxicity. Other tree species were intermediate in sensitivity. In other tests, Bt mixes were held for up to 23 days under outdoor, greenhouse, freezer and laboratory conditions. Generally, by 23 days, the activity of the mixes had declined slightly and this was most evident in samples held frozen. Apparently, these mixes can be held for eight days with only minor loss of activity.

Numerous Bt weatherability evaluations were done and it was again shown that formulations without added sticker are very easily washed off foliage by small amounts of rain. This can be remedied by adding certain stickers; not all stickers are effective, however.

Field tests in Pennsylvania concerned evaluation of several formulations of Bt, particularly SAN-415 and ABG-6163. Additionally, Thuricide 48LV was applied at various times such that the target larvae were in second, third, fourth, or fifth instar. Unfortunately, a dramatic population collapse in the area compromised the evaluations. However, anecdotal observations suggest that the new Bt strain performs satisfactorily in the field and that applications of 16 BIU per acre are effective in controlling later instar larvae. These results should not be interpreted to mean that Bt can be used at optimum effectiveness against large larvae. Prudence still dictates that sprays be aimed at the smaller and more sensitive second and third instar larvae.

Laboratory and field tests worked out a method of aerially releasing gypsy moth eggs in connection with the sterile male program. Twenty stickers were tested for their effect on egg hatch; it was invisioned that it may be necessary to retain the aerially dropped eggs in the canopy to facilitate location of foliage and establishment of feeding by larvae. Several stickers were identified which were suitable. The egg dispensing apparatus was calibrated in the laboratory and tables developed calibrating egg delivery at various auger speed settings. The equipment was also tested in the field, and was found to perform satisfactorily.

Mating disruption demonstrations in Oconomowoc, Wisconsin and Carteret County, North Carolina have given equivocal results on the effectiveness of the technique. In fact, Carteret County data suggest that the method is not workable for eradicating sparse populations; however, those conclusions must be tempered by the clear observation that the formulation applied to the project area weathered very poorly. Presently there are no other mating disruption demonstrations in progress or planned, and the ultimate fate of this approach for handling isolated gypsy moth infestations is dubious.

It has been observed that Hercon flakes used in pheromone disruption trials do not adhere to foliage very effectively and, only a few days after application, the once abundant pheromone-containing flakes, are difficult to find. Several sticker mixes were evaluated and it was shown that the presently used stickers are relatively ineffective in adhering flakes to foliage in the presence of rain. The latex sticker from Monsanto, RA-1990, clearly improves the adhesion of flakes to foliage, and registration details should be attended such that the sticker can be used in future applications.

There has been increased interest in the use of mass trapping for eradicating isolated infestations. Field tests have shown that mating can be prevented by the placement of 25 traps per hectare. Observations showed that in order for mating to be reduced, males must be trapped more quickly than they locate and mate with females. Females tend to mate later in the day, and males tend to be captured in traps earlier. It was also discovered that, in the absence of traps, many males multiple-mate with females and some males were observed to mate with up to four different females. Conversely, in mass trapped plots, multiple mating was rare. This indicates that trapping reduces mating because it minimizes the time that males have available to multiple mate.

A demonstration project in Monona, Wisconsin is indicating that that population can be eliminated solely through the use of mass trapping. Calculations suggest that no successful mating occurred in 1984. Of course, 1985 trapping results will attest to the accuracy of that prediction.

Several formulations of (+) disparlure from Albany International (currently Pest Select) were tested and found to be inferior to the presently used Hercon formulation. Thus, Hercon stands as our sole supplier of trap baits for the gypsy moth survey program. Interestingly, in 1981, 82, and 83, pheromone dispensers that had been held in the greenhouse for seven days captured more males than those dispensers taken directly from the storage pack and bioassayed. Curiously, that aging treatment had no effect on the 1984 lot; we apparently don't understand everything that makes these controlled release devices tick. Nevertheless, they are widely used for gypsy moth survey and other tests reported described simple methods of installing the dispensers into traps.

In the course of bioassaying various combinations of pheromones, it was found that the pheromone components of the summer fruit tortrix moth, Adoxophyes orana, were strongly inhibitory to gypsy moth when added to disparlure-baited traps. Small scale disruption tests were run to determine the effectiveness of the Adoxophyes orana pheromone as a disruptant of gypsy moth mating behavior. Applied at 40 grams per hectare, Adoxophyes orana pheromone had no effect on gypsy moth trap catch. Disparlure applied at that rate reduced trap catch about 80%. Thus, the inhibitory effect of Adoxophyes orana pheromone to gypsy moth, when incorporated into disparlure-baited traps was not manifested when broadcast in the area containing gypsy moth traps. Behavioral observations around traps containing both disparlure and Adoxophyes pheromone indicate that the inhibitory effect of Adoxophyes compounds occurs up to one meter away from the trap; however, effects are still observed at the entry port.

The sterile male project field demonstration phase was basically initiated in 1980 with the release of fully sterile insects in Berrien County, Michigan. After three consecutive years of release of fully sterile adults, the population there has declined to undetectable levels and has remained that way for two seasons. Accordingly, we conclude that eradication has been achieved through the release of fully sterile adults.

In another field demonstration project in Horry County, South Carolina, "partially sterlized" males were released into a small isolated infestation. The strategy involved releasing males which, when they mated with normal females, yielded a second generation of fully sterile adults the following season. The release of partially sterile insects was followed by successful detection of fully sterile adults the following year. Evidently, no reproduction took place, and that infestation also is considered eradicated. A similar trial was conducted in Kent County, Maryland, and because populations were higher in Maryland sampling was more intensive. The results suggest that the release of partially sterile males caused populations to remain stable, whereas in the control plots, populations increased dramatically. Evaluation of these release areas is continuing.

The release strategy for the sterile male project has evolved into an egg release system. This variation of the technique involves mating partially sterilized males in the laboratory with normal females. Those resultant egg masses hatch and larvae develop into sterile adults. Clearly, by releasing these egg masses into the target populations, the logistics of sterile insect release are greatly simplified. Only one release per year is necessary and the sterile population should develop in synchrony in the field with the target insects. Currently, most development work is focusing on this approach. The first release of this nature was conducted in Maryland and results are rather preliminary. However, it was clearly shown that sterile insects can be detected in the release areas following an egg release, and that their occurance is synchronous with the native population in the area. Concomitant with these trials are numerous behavioral assessments of F_1 individuals. Since released insects must compete for resources in the field, their competitive fitness is of paramount significance. Numerous experiments have revealed no significant behavioral deficiencies.

The gypsy moth rearing facility reached new levels of production this year with over 6.5 million eggs infested onto diet, and life stages provided to numerous cooperators in the U.S. This was also the first year of large scale F_1 egg production, and during the period July 23, 1984 - January 10, 1985, 650,000 egg masses were produced for subsequent release into test plots in the summer of 1985.

Our monitoring of the colonies again revealed no dramatic changes in insect performance. However, we have encountered a condition commonly referred to as "straggling" which has subsequently been found to be caused by rickettsia infection. Insects thus afflicted typically live for a long time but fail to feed and develop. Thus, pupal yield can be dramatically reduced when rickettsia incidence is high.

In an effort to streamline F_1 egg production, several innovations have been developed and implemented. A conveyor belt system has been incorporated into the pupal collection area which facilitates harvesting and sexing of pupae. Various egg mass dehairing devices were tested and a large scale system adopted for use. Of greatest potential impact however, is defining the rearing conditions which provide the widest possible rearing window for eggs. Obviously, eggs that are to hatch in the spring can only be produced over a certain period of time. If they are chilled too long or not long enough, reduced hatch is a problem. Thus, we have been evaluating different embryonation and chilling temperatures to determine conditions which maximize the length of time over which these eggs can be produced and stored.

Other assessments of growth and development of the colonies show that about 88% of the male insects in NJSS are five instar type. The remaining 12% are distributed between four, six, and seven instar types. Females, on the other hand, are approximately 67% five instar type and 28% sixth instar type; .3% and 4.6% of females pupaed after four and seven instars, respectively. The shift from six to five instar type females has been occuring over a period of time and with the selection procedures in place, we would expect that five instar type females will become more common in future generations.

Tests were conducted with the Phoenix Methods Development Laboratory to develop a method for marking gypsy moth eggs with strontium chloride. A procedure was devised which can be used for producing eggs that contain detectable levels of strontium chloride. This label persists above background for the first two or three days of larval development; thereafter, it is presumably excreted and the tag is lost. However, insects thus labeled would be useful for determining the dispersal distance of first instar larvae.

A project addressing the biological and economic ramifications of redistributing alfalfa weevil parasitoids has been ongoing since 1981, and is documenting the changes in parasite distribution and abundance following parasite redistribution. Evaluations are conducted at 120 fields located from New Jersey to Nebraska. Particularly in Ohio, where the Bathyplectes curculionis is widely distributed, it appears that Bathyplectes anuras is replacing B. curculionis as the former moves into the area. The consequenc of this displacement is not clear, but B. anuras is the dominant larval parasitoid in the east, where populations tend to be maintained at very low levels. Regression testing has revealed a good inverse relationship between percent parasitism by Microctonus aethiopoides and alfalfa weevil density. These data suggest that high levels of Microctonus aethiopoides parasitism are associated with low levels of alfalfa weevil.

Techniques have been worked out for using electrophoresis for distinguishing between eastern and western strains of alfalfa weevil. The technique requires careful physiological aging of specimens. Western weevils express the diagnostic enzyme two to three weeks sooner than eastern weevils.

Releases of the parasite Pediobius foveolatus were made into Mexican bean beetle populations over large areas in Ohio. Parasite establishment was achieved in most nurse plots receiving releases and parasite movement to the surrounding soybean fields was quite consistant. These release areas are being evaluated in 1985 to determine if subsequent year populations are lowered due to the parasite release. Theoretically, high levels of parasitism lower overwintering adult populations with consequent lower infestation levels the following year.

The efforts to develop serological methods for distinguishing between fungal pathogens of corn were unsuccessful, and have been discontinued. Because of program leadership influence, we have put recent effort into developing improved output reports from the Fort Collins Computer Center (FCCC) and developing a system for standardizing survey procedures that are used nationally. These projects are proceding satisfactorily and should contribute to the effectiveness of the centralized reporting system fundamental to the Cooperative National Plant Pest Survey Detection Program.

Extensive efforts have been made to enhance the use of pheromones for detecting possible introductions of exotic pests in the United States. The work has principally focused on evaluating traps and pheromone dispensers for exotic pests and determining the potential for combining two (or more) pheromones in a single trap. Of particular interest is the opportunity to incorporate exotic pheromones into traps that are already widely placed in this country (such as gypsy moth, pink bollworm traps). Results of tests conducted in the United States, Republic of South Africa, Australia, and France on about fifteen different species show, not surprisingly, that many pheromone combinations are incompatible. That is, one of the two target species is inhibited by the other species pheromone. However, we have identified seven combinations which are perfectly compatible for simultaneous survey. This work has led to the placement of several thousand traps for six exotic species in thirty-six states. Thus far, no exotic pests have been detected. A questionaire was also distributed that gathered information on the dimensions of trapping done for insects in this country. Forty-eight states responded and sixty-eight different species were surveyed for with over 690,000 traps. Tortricids and noctuids were the most heavily surveyed families. These data are useful to identify ongoing trapping programs that might lend themselves to incorporation of exotic pheromones. Also included in this report are the recommendations prepared and distributed to cooperating states for exotic pest detection. The recommendations include brief descriptions of life history and biology, trapping procedures and maps showing potential geographical range in the United States.

PESTICIDE TESTING SECTION

PHEROMONE, BEHAVIOR AND ECOLOGY SECTION

PHEROMONE, BEHAVIOR AND ECOLOGY SECTION

PHYSIOLOGY AND MASS REARING SECTION

BIOCONTROL SECTION

Project Number: GM 6.1.5
Project Title: Regulatory Treatments
Report Period: October 1, 1983 - September 30, 1984
Report Type: Interim
Project Leaders: W.H. McLane and J.A. Finney

The main objective of this work is the development of new and improved treatments for regulated items moving from quarantined areas to non-infested locations. This project is primarily directed toward the development of treatments for recreational vehicles, mobile homes and outdoor household articles.

During the past year, several companies demonstrated interest in developing home-owner treatments such as oil-based ovicides for egg masses, direct spraying of larvae, and placement of treated tree bands and/or natural barriers. Some of these techniques could be useful in our regulatory program.

In the laboratory, Spray N Kill, when diluted by distilled water, is effective in killing eggs. Field tests have substantiated these results. However, a question was raised as to whether these results will be affected by water pH. Accordingly, the following test was conducted.

Spray N Kill mixtures of the active ingredient and water of varying pH's were formulated by combining the commercial product with distilled water, the pH of which had been adjusted with phosphoric acid or potassium hydroxide. Egg masses were treated to the point of run off with a hand sprayer. Hatch in controls was expected to begin in 3-5 days. Three egg masses were treated with each mixture. All egg masses were held individually in petri dishes for 2 weeks incubation in an environmental chamber at 80°F, 60% RH after which, hatch counts were made. The spray mixtures were aged in the laboratory at 70°F.

Table 1. The pH of the original water used for each mix and resulting pH of each mix over a period of time.

Spray N Kill used oz./gal.	Original pH of water used	Days mix aged in laboratory							
		0	3	6	8	10	16	23	30
1.0	4	3.2	3.0	2.9	2.9	2.9	2.9	2.8	2.7
	5	3.2	2.9	2.9	2.9	2.9	2.8	2.7	2.6
	6	3.2	3.0	2.9	2.9	2.9	2.8	2.7	2.7
	7	3.3	3.0	3.0	3.0	3.0	2.9	2.8	2.7
	8	3.4	3.1	3.0	3.0	3.0	2.9	2.8	2.7
	9	3.3	3.1	3.0	3.0	3.0	2.9	2.8	2.7
	10	3.4	3.1	3.0	3.0	3.0	2.9	2.8	2.7
2.0	4	2.9	2.7	2.6	2.6	2.6	2.5	2.4	2.4
	5	2.9	2.7	2.6	2.6	2.6	2.6	2.4	2.4
	6	2.9	2.7	2.6	2.6	2.6	2.5	2.4	2.4
	7	2.9	2.7	2.6	2.6	2.6	2.5	2.4	2.4
	8	3.0	2.7	2.6	2.6	2.6	2.5	2.4	2.4
	9	2.9	2.7	2.6	2.6	2.6	2.5	2.4	2.4
	10	3.0	2.7	2.6	2.6	2.6	2.5	2.4	2.4
3	4	2.6	2.4	2.4	2.4	2.4	2.3	2.2	2.2
	5	2.7	2.5	2.4	2.4	2.4	2.3	2.2	2.2
	6	2.7	2.5	2.4	2.4	2.4	2.3	2.2	2.2
	7	2.7	2.5	2.4	2.4	2.4	2.3	2.2	2.2
	8	2.7	2.5	2.4	2.4	2.4	2.3	2.2	2.2
	9	2.7	2.5	2.4	2.4	2.4	2.3	2.2	2.2
	10	2.7	2.5	2.4	2.4	2.4	2.3	2.2	2.2

Viable egg masses were treated with each dosage and mix on all dates listed in Table 1. No hatch was observed from any treated egg mass. Checks had hatch of 80-90%. It appears that the pH of the mix has no effect on the activity of Spray N Kill. Because of the acidic nature of Spray N Kill, a mix is quickly rendered acidic regardless of the pH of the diluent used.

In a similar test, Spray N Kill was mixed with water with a resulting drop in pH taking place. Mixes were then adjusted up to pH 4 through 10. Eggs were treated with each mix and no hatch occurred after incubation for 7 days.

Five samples of d-limonine, a citrus extract, were tested in the laboratory. Five egg masses were treated with each sample using 1.5 ml per mass. After 7 days, no hatch had occurred from any treated mass. Hatch in untreated checks was 80-90%. This material will be field tested during our 1984-1985 field trials.

A series of emulsifiers were tested on viable egg masses in the laboratory using a 50% concentration. Materials were diluted with isopropyl alcohol. Three egg masses were treated with each material to the point of run-off. The material was applied with a glass pipette.

Table 2. Percent hatch of gypsy moth eggs 7 days after treatment with various emulsifiers.

Material	Percent hatch	Material	Percent hatch
ADSEE-815	0	Sponto-6524	0
ADSEE-801	0	Witgonate-A0S	25
ADSEE-799	0	Witgonate-79S	70
ADSEE-775	1	Witgonate-SE5	0
EMCOL-4500	3	Witgonate-DS10	5
EMCOL-CC-9	0	Witgonate-P1059	0
EMCOL-CC-42	0	Witgonate-605T	0
EMCOL-CC-55	0	Witconol-H31A	0
EmpHos-CS-131	2	Witconol-NP80	0
EmpHos-CS-147	5	Witconol-NP120	2
EmpHos-PS-121	0	Witconol-1206	0
EmpHos-PS-33	15	Check/Alcohol	0 1/
Sponto-168D	6	Check/Water	90-100
Sponto-H3A	0	Check/Untreated	90-100

1/ The reduction of hatch observed with all materials may have been due to the diluent instead of the test material.

There is a need for a treatment to effectively control the hatch of gypsy moth eggs in regulatory situations. Compounds such as creosote that have been used effectively in the past but may not be available for use in the near future. Laboratory and field tests during the past 5 years have identified a number of materials that are effective when applied to egg masses. However, most materials that are efficacious in the laboratory have proven to be ineffective in the field. Furthermore, agents must be effective regardless of the time of the year (stage of the egg) they are applied.

During 1983-1984, sixteen ovicidal materials were field tested near Otis Air National Guard Base, Massachusetts. Starting in late September, 10 egg masses were treated with each material using small hand sprayer. Egg masses were treated to the point of complete saturation. Egg masses were treated on a monthly schedule until the last application was made April 9, 1984. Treated egg masses were left in the field until collected for hatchability tests in mid-April.

Egg collection was accomplished using 2 techniques. Five intact masses from each treatment date were collected into plastic petri dishes. Plugs of approximately 50 eggs each were removed from each of the 5 remaining masses. Untreated masses were collected at 2 locations within the study area for use as a check.

All egg masses were incubated in the laboratory at 27^{o}C with 60% RH. After 10 days, percent hatch was estimated in dishes with complete masses. All eggs from each plug sample were counted and checked for hatch to give an accurate measurement of hatch.

Table 3 - Percent hatch of gypsy moth egg masses treated with various materials between September and April 1984. Values are the average of 5 observations.

Material	Percent mix	Percent hatch as indicated by date treated							
		9/27 hatch	10/3 hatch	11/8 hatch	12/2 hatch	12/22 hatch	1/5 hatch	3/9 hatch	4/9 hatch
Light water	100	0	4	12	71	82	90	82	0
Top Job	100	--	81	84	86	77	90	90	0
Pine Scent	100	1	1	11	88	--	15	20	0
Triton X-100	50	84	85	100	90	--	93	--	17
Soybean Oil	50	0	0	0	0	0	0	0	0
AK-1695	50	1	75	--	--	--	--	--	--
H-44C	50	0	65	85	80	41	67	94	0
Insecticide Soap	50	4	50	100	54	80	25	100	0
Spray N Kill	2 oz/g	90	39	100	90	100	4	95	0
Spray N Kill	4 oz/g	75	70	60	90	24	0	20	0
Pounce	.06 lbs/g	0	50	30	0	0	0	0	0
Pydrin	.06 lbs/g	1	40	10	0	0	5	1	0
Dimilin	.06 lbs/g	90	100	100	90	85	100	94	94
Zapper	100	0	0	0	5	0	--	0	0
KCOPO	100	69	80	98	--	40	--	--	0
TCOPO	100	60	3	20	40	75	21	--	0
Norpine	100	0	3	2	0	0	7	--	0
Check		100	100	100	100	100	100	100	100

Zapper (containing creosote), soybean oil and Norpine were effective in preventing hatch throughout the treatment period. Pounce and Pydrin were effective over the last half of the treatment period. Spray N Kill, a registered egg mass treatment, gave poor results even when used at twice the recommended dosage.

With materials such as Dimilin, Pounce and Pydrin, hatch is not affected but larval mortality takes place as they emerge from the egg case and contact the material. Larvae from egg masses treated with Dimilin were placed onto artificial diet and 100% mortality occurred in 8 days.

Based on the results of this study, it is recommended that soybean oil and Norpine be used for the treatment of gypsy moth egg masses in regulatory situations. Norpine is available from Northwest Petrochemical Corporation, Anacortes, Washington. Soybean oil can be purchased at supermarkets or grain mills.

Spray N Kill and Zapper were tested to determine what effect they might have on car paint finishes. Using various dilutions of each material, car paint plates were sprayed in a laboratory spray chamber and allowed to dry for 48 hours. Treated plates were then exposed to 4 types of cleaning and then checked under a microscope for pitted or faded paint. Six paint colors were used in the test. Surfaces were washed with water only, water and Mr. Clean, brushed with water and Mr. Clean, and 15 minute soaking followed by wash with water and Mr. Clean.

Spots were completely visible on all treated surfaces regardless of the clean-up technique used. Spots were not visible on check plates.

Table 4. Applications of Zapper and Spray N Kill applied to car finishes.

Material	Dosage	Paint Color
Spray N Kill	2.0 oz./gal	Beige
Spray N Kill	4.0 oz./gal	Pastel Blue
		Yellow
Zapper	5%	Light Brown
Zapper	10%	Firemist
Zapper	25%	Red
Zapper	50%	
Zapper	75%	
Check	-	
Check/Kerosene	-	

Tests were conducted with a rubber type tree band material supplied by David Harlow Co.. Virus and Bacillus thuringiensis was applied to tree bands to see if gypsy moth larvae would spread the material onto the foliage and then consume it.

Eight isolated oak trees were selected near Otis ANGB, Massachusetts for conducting tree band studies. One-thousand laboratory reared larvae were released on each tree. Three drop cloths (3 x 3') were placed under each tree to collect frass. After larvae were established on test trees (2 days) a single rubber band, approximately 6" in diameter was placed around the trunk of each tree below the lowest limb. A burlap band was placed around each tree trunk below the treated rubber bands. Three bands were then treated completely with Bacillus thuringiensis and 3 were treated with virus, with the remaining 2 acting as untreated checks. A lip on each band was placed at the top to shield the sprays from adverse weather. The normal migration of the larger larvae allowed them to come in contact with the material on each band. It was considered that the materials might be mechanically spread to foliage and eventually eaten with mortality being the end result.

Frass on each drop cloth was collected and weighed on a daily basis. Following treatment, larvae were collected on a daily basis and reared on artificial diet to check mortality. Dead larvae were also counted on each drop cloth and under burlap bands.

Table 5. Amount of frass collected on 3 drop cloths under each tree.

Treatment	Grams of frass pretreatment in 24 hours	Grams of frass posttreatment in 8 days	Percent change
BT	3.09	8.99	+191
NPV	1.22	4.0	+227
Check	1.88	3.8	+102

Table 6. Larval mortality resulting from each treatment.

Treatment	Total mortality drop cloths/ burlap bands	Live larvae collected	Percent mortality of collected larvae on diet after 8 days
BT	65	222	74
NPV	6	143	54
Check	11	84	56

Based on frass weighings, larvae on band treated trees actually increased feeding when compared with untreated checks, indicating no control. Larval mortality readings on drop cloths under burlaps, and on diet indicate a possibility of some effects from Bacillus thuringiensis.

Project Number: GM 8.1.3
Project Title: Laboratory Screening of Candidate Pesticides and Microbials Against the Gypsy Moth
Report Period: October 1, 1983 - September 30, 1984
Report Type: Interim
Project Leaders: W. H. McLane and J. A. Finney

The objectives of this laboratory screening project are to collect and evaluate mortality data on experimental and registered compounds potentially useful for gypsy moth control, and to select materials for field studies and further development. These tests are designed to identify new materials and to increase the effectiveness of registered products.

Unless otherwise stated, all tests have been conducted with our standard red oak seedling technique. Test insects are of the New Jersey strain and have been laboratory reared on artificial diet.

During 1984, a number of State and Federal agencies used Bacillus thuringiensis to treat gypsy moth infestations. In all, more B. thuringiensis was used in 1984 than any previous year. In most cases, single applications of 12 BIU/acre were very effective in controlling defoliation.

In isolated infestations, multiple applications of B. thuringiensis was the principle treatment used. Post-spray trapping in and around treatment areas indicated good control was achieved. Single and multiple applications of Dipel, Thuricide and Bactospeine resulted in little if any problems with phytotoxicity or car finishes. All formulations mixed and handled well.

During this reporting period, laboratory studies continued to be directed toward the development and improvement of biological insecticides for gypsy moth control.

A number of laboratory studies were conducted with a new strain of B. thuringiensis called NRD-12. In laboratory studies Dr. DuBois has found this strain to be consistently more effective than the HD-1 strain presently used by most formulators. The new strain is presently being supplied by Zoecon for experimental use and is identified as SAN-415 (32 BIU/gal). Abbott's formulation is identified as ABG-6163 (48 BIU/gal).

Table 1. Percent mortality of 2nd instar gypsy moth larvae after a 4 day exposure to foliage treated with 3 B. thuringiensis formulations.

Dosage BIU/ gal/acre	Thuricide 48LV		Thuricide 64BX		SAN-415	
	mortality	defoliation	mortality	defoliation	mortality	defoliation
4	62	15	67	17	84	10
8	83	4	95	3	90	4
12	86	3	99	4	96	3
Check	0	100	0	100	0	100

Table 2. Percent mortality of 2nd instar gypsy moth larvae after a 4 day exposure to foliage treated with 2 B. thuringiensis formulations.

Dosage	Thuricide 48LV		SAN-415	
BIU/gal/acre	mortality	defoliation	mortality	defoliation
1.0	29	32	19	40
2.0	65	7	65	27
3.0	68	6	98	3
4.0	73	5	91	4
8.0	80	4	87	4
Check	0	95	0	95

Table 3. Percent mortality of 2nd instar gypsy moth larvae after a 4 day exposure to foliage treated with 2 B. thuringiensis formulations at 12.0 BIU/96 oz/acre and weathered with various amounts of rainfall.

Inches	Thuricide 48LV		SAN-415	
rain	mortality	defoliation	mortality	defoliation
-	78	3	95	4
0.1	78	3	69	15
0.2	91	3	40	36
0.3	60	6	39	33
0.4	78	8	40	38
0.5	64	14	74	18
Check	0	100	0	100

Table 4. Percent mortality of 2nd instar gypsy moth larva after a 4 day exposure to foliage treated with 2 B. thuringiensis formulations at a rate of 96 ounces per acre.

Dosage	Thuricide 32LV		SAN-415	
BIU/acre	mortality	defoliation	mortality	defoliation
.25	4	81	10	83
.5	9	71	28	71
1.0	32	33	65	23
2.0	53	27	91	14
4.0	75	15	98	5
Check	0	100	0	100

Table 5. Percent mortality of 2nd instar gypsy moth larvae after a 3 day exposure to oak foliage treated with B. thuringiensis using 20 oz. per acre and applied in small and large drops.

Material	Dosage BIU/acre	Large drops		Small drops	
		mortality	defoliation	mortality	defoliation
Dipel 4L	2.0	61	37	33	49
	4.0	83	15	46	32
Thuricide 48LV	2.0	59	44	45	36
	4.0	52	41	62	32
SAN-415	2.0	43	34	62	34
	4.0	77	25	87	18
Check	-	0	99	0	99

Table 6. Percent defoliation of 1.0 inch dia. oak foliage discs treated with B. thuringiensis and exposed to 2nd instar gypsy moth larvae in a paired choice test for a 24 hour period.

T32LV	Check	T48LV	Check	SAN-415	Check	Dipel 4L	Check	Dipel 6L	Check
12	40	13	64	10	41	6	59	16	52

Table 7. Percent defoliation of 1.0 inch dia. oak foliage discs treated with B. thuringiensis and exposed to 2nd instar gypsy moth larvae in a 6-way choice test for a 24 hour period.

T32LV	T48LV	SAN-415	Dipel 4L	Dipel 6L	Check
5	3	2	2	1	80

Table 8. Percent mortality of 2nd instar gypsy moth larvae after a 3 day exposure to oak foliage treated with B. thuringiensis using 96 oz. per acre.

Material	Dosage BIU/acre	Mortality	Defoliation
San-415	4.0	82	6
	2.0	65	13
	1.0	43	26
Thuricide 48LV	4.0	81	4
	2.0	53	13
	1.0	28	28
Dipel 4L	4.0	31	38
	2.0	26	50
	1.0	2	71
Dipel 6L	4.0	17	53
	2.0	6	63
	1.0	1	77
Check	-	0	100

During 1983 there were reports of phytotoxicity on some species of plants in areas treated with the oil base Dipel formulation of B. thuringiensis. This occurred mainly in Washington where Dipel 6L was applied using a 50/50 mix.

Laboratory studies found 3 B. thuringiensis formulations to be phytotoxic to the foliage of a number of tree species.

Table 9. Degree of phytotoxicity on foliage of 15 tree species treated with Dipel 6L, Dipel 8L and Thuricide 64BX, 48 hours prior to visual insection.

Tree	Dipel 6L				Dipel 8L				Thuricide 64BX			
Species	N	L	M	H	N	L	M	H	N	L	M	H
Poplar				X				X				X
Red oak		X				X				X		
White oak			X				X				X	
Pine		X								X		
Plum	X				X				X			
Lilac			X				X				X	
Catalpa				x				x				x
Blueberry			X					X				X
Pear		X					X					X
Birch				X				X				X
Maple				X				X				X
Cucumber	X				X				X			
Peach	X				X				X			
Locust	X				X				X			
Ash	X				X				X			

N - no evidence of burning
L - light burning and droplets vague
M - burning easy to see and very obvious
H - extensive burning and leaf curl

Above test results based on a dilution of 1.0 part BT. and 1.0 part water.

Table 10. Mortality of 2nd instar gypsy moth larvae 5 days after placement on oak foliage treated with 8 BIU of 3 B. thuringiensis formulations held under various climatic conditions for various periods of time.

Material	Environmental condition	Percent mortality days in storage			Percent defoliation days in storage		
		0	8	23	0	8	23
Bactospeine	Outside	96	98	80	10	12	17
Bactospeine	Greenhouse	92	89	85	11	12	19
Bactospeine	Freezer	90	88	80	10	19	22
Bactospeine	Freezer (con)	92	100	71	10	10	21
Bactospeine	Laboratory	90	96	95	10	11	10
Thuricide 32LV	Outside	100	100	97	5	6	14
Thuricide 32LV	Greenhouse	98	98	90	5	5	12
Thuricide 32LV	Freezer	98	98	91	10	10	20
Thuricide 32LV	Freezer (con)	98	99	82	10	14	22
Thuricide 32LV	Laboratory	95	95	80	10	8	14
Dipel 4L	Outside	98	98	87	5	6	9
Dipel 4L	Greenhouse	98	96	94	10	10	5
Dipel 4L	Freezer	95	97	97	10	11	19
Dipel 4L	Freezer (con)	96	84	51	10	21	28
Dipel 4L	Laboratory	95	95	82	10	11	12
Check	-	0	0	0	100	100	100

Table 11. Percent mortality of 2nd instar gypsy moth larvae exposed to oak seedlings treated with various 12 BIU undiluted Thuricide formulations.

Thuricide formulation	Percent mortality Inches rain				Percent defoliation Inches rain			
	0.0	0.5	1.0	2.0	0.0	0.5	1.0	2.0
26B-A	90	60	46	16	6	21	26	52
24B-B		52	46			14	19	
24B-C		91	83			6	5	
24B-D		57	68			20	13	
24B-E		85	86			8	5	
24B-F				88				10
24B-G				68				21
24B-H				50				41
24B-I				53				38
32B-A		19	3			61	80	
32 LV		9	11			76	78	
Check		9	0	0		100	100	100

Table 12. Percent mortality of 2nd instar gypsy moth larvae exposed to oak foliage treated with SAN-415, Thuricide 32LV and Thuricide 48LV, using 6 BIU/96 oz/acre.

Formulation	Percent mortality	Percent defoliation
SAN-415 Lab. Sample	90	7
SAN-415 Field Sample	84	15
Thuricide 32LV	63	20
Thuricide 48LV	68	20
Check	0	100

Percent mortality and defoliation are an average of 3 separate tests.

Table 13. Percent mortality of 2nd instar gypsy moth larvae exposed to oak seedlings treated with Thuricide and Wilt-Pruf and then exposed to rainfall. Application was 12 BIU/96 oz/acre.

Thuricide formulation	Sticker	Percent mortality Inches rain			Percent defoliation Inches rain		
		0.0	1.0	2.0	0.0	1.0	2.0
48LV	None	71	64	21	7	9	50
48LV	3% Wilt-Pruf		67	83		9	7
32LV	None	86	18	2	3	29	54
32LV	3% Wilt-Pruf		17	25		22	34
Check		0			100		

Table 14. Percent mortality of 2nd instar gypsy moth larvae after a 3 and 5 day exposure to oak foliage treated with B. thuringiensis.

Material	After 3 days				After 5 days			
	2 BIU		4 BIU		2 BIU		4 BIU	
	Mor	Def	Mor	Def	Mor	Def	Mor	Def
ABG-6163	12	53	27	37	44	72	56	51
Dipel 4L	4	55	14	41	18	86	43	58
SAN-415	55	17	85	4	92	24	97	7
Thuricide 32LV	41	19	43	10	70	29	82	20
Check	0	88	0	88	0	100	0	100

Table 15. Percent mortality of 2nd instar gypsy moth larvae after a 4 day exposure to oak foliage treated with Bactospeine and exposed to rainfall.

Inches rain	12 BIU/96 oz/acre		12 BIU/gal/acre	
	mortality	defoliation	mortality	defoliation
-	76	15	96	3
1.0	1	64	18	44
0.5	20	68	48	41
0.2	25	58	36	51
0.1	55	34	40	53
Check	0	100	0	100

Table 16. Percent mortality of 2nd instar gypsy moth larvae after a 4 day exposure to oak foliage treated with Futura and exposed to rainfall.

Inches rain	12 BIU/96 oz/acre		12 BIU/gal/acre	
	mortality	defoliation	mortality	defoliation
-	76	15	96	9
1.0	13	60	5	90
0.5	12	60	35	48
0.2	9	41	52	38
0.1	49	33	40	35
Check	0	100	0	100

Table 17. Percent mortality of 2nd instar gypsy moth larvae after a 3 and 6 day exposure to oak foliage treated with Thuricide 32LV and Poly Control and exposed to rainfall.

Amount Poly Control	Inches rain	3 Day Reading		6 Day Reading	
		mortality	defoliation	mortality	defoliation
None	-	10	26	100	32
None	0.2	9	42	83	64
2.0 oz/100 g	-	31	18	100	22
2.0 oz/100 g	0.2	16	40	89	50
1.0 oz/100 g	0.2	23	23	87	40
0.5 oz/100 g	0.2	10	22	84	50
Check	-	1	100	9	100

Table 18. Percent mortality of 2nd instar larvae 3 days after placement onto oak seedlings treated with 8 BIU/acre of Bactospeine, Dipel 4L and Thuricide 32LV and various stickers and then exposed to 1.0 inches of rain.

Sticker	Bactospeine	Dipel	Thuricide
(no sticker, no rain)	29	41	32
(no sticker)	1	0	2
1% Clear Spray	4	1	4
3% Clear Spray	22	2	8
3% Exhalt-800	0	3	15
3% Exhalt-4-10	2	1	4
3% Wilt-Pruf	8	1	22
Check	0	0	0

Table 19. Percent mortality of 2nd instar gypsy moth larvae after a 3 day exposure to oak seedlings treated with Terminate and Dipel 4L.

Dosage	TERMINATE		DIPEL 4L	
BIU/gal/acre	mortality	defoliation	mortality	defoliation
16	80	12	95	5
12	72	17	94	3
8	67	15	90	4
4	57	35	24	47
2	45	48	18	58
Check	0	100	0	100

Table 20. Percent mortality of 2nd instar gypsy moth larvae after a 4 day exposure to oak foliage treated with 3 B. thuringiensis formulations.

Material	Dosage/Rate BIU/acre	Percent mortality after 4 days	Percent defoliation
Terminate	4.0	30	49
Terminate	8.0	62	27
Terminate	12.0	64	31
Thuricide 48LV	4.0	70	14
Thuricide 48LV	8.0	80	12
Thuricide 48LV	12.0	89	8
Dipel 6L	4.0	12	59
Dipel 6L	8.0	38	42
Dipel 6L	12.0	76	18
Check	--	0	100

Table 21. Percent mortality of 2nd instar gypsy moth larvae after a 4 day exposure to oak seedlings treated with B. thuringiensis and then exposed to various amounts of rainfall.

Material	Dosage/Rate BIU/acre	Inches rain	Percent mortality after 4 days	Percent defoliation
Terminate	8.0	-	42	35
Terminate	8.0	0.1	49	40
Terminate	8.0	0.2	58	38
Terminate	8.0	0.25	17	22
Terminate	8.0	0.3	66	46
Terminate	8.0	0.5	17	29
Terminate	8.0	1.0	0	92
Terminate	8.0	2.0	0	91
Dipel 4L	8.0	-	30	46
Dipel 4L	8.0	0.25	16	66
Dipel 4L	8.0	0.5	2	81
Dipel 4L	8.0	1.0	0	98
Dipel 4L	8.0	2.0	0	98
Thuricide	8.0	-	75	7
Thuricide	8.0	1.0	9	46
Thuricide	8.0	2.0	4	77
Terminate	12.0	-	86	14
Terminate	12.0	0.1	68	42
Terminate	12.0	0.2	65	30
Terminate	12.0	0.3	58	36
Terminate	12.0	0.5	19	68
Check	-	-	0	100

The citrus extract d-limonine was tested against 2nd instar gypsy moth larvae using the standard oak seedling technique. After a 5 day exposure no mortality occurred.

Orthene 75S and Sevin XLR were tested on isolated trees to establish their residual effect on late instar gypsy moth larvae. During July and August, isolated oak and apple trees were infested with 1,000 laboratory reared gypsy moth larvae, half being 4th instar and the remainder 5th instar. Larvae were placed in a one gallon container that was then tacked to the side of the tree. Larvae were allowed to climb from the container onto the tree. Three drop cloths (3 x 3') were then placed on the ground under each tree. Six trees were used for treatment and 2 for a check. After a 2 day period a significant amount of frass was being collected on each drop cloth indicating establishment of larvae onto the tree. Treatment trees were then sprayed with a back-pack ground sprayer using approximately 1 gallon of spray volume per tree. Orthene was mixed using 10.5 ounces of material per 100 gallons of water. Sevin was mixed using 0.5 ounces per gallon. Following treatment, frass was collected and weighed and mortality on drop-cloths was recorded on a daily basis. Foliage was removed from treated and untreated trees daily and was bioassayed in the laboratory using III, IV and V instar gypsy moth larvae.

Table 22. Average grams of gypsy moth frass collected on drop cloths under trees treated with Orthene 75S on August 22, 1984.

	Average grams of frass			
DATE:	8/22	8/24	8/26	8/28
Treatment	0.795	0.091	0.073	0.035
Check	0.52	1.875	0.165	0.19

Table 23. Average number of dead larvae collected on drop cloths under Orthene treated trees over a 5 day period following treatment.

Treatment	Check
27	.5

Table 24. Percent mortality of gypsy moth larvae fed foliage treated with Orthene 75S on August 22, 1984.

	Percent mortaltiy					
	After 24 hours			After 48 hours		
Bioassay	Instar			Instar		
date	III	IV	V	III	IV	V
8/22	95	88	87	100	90	93
8/23	73	50	92	95	87	95
8/24	70	85	73	90	98	85
8/25	77	63	78	82	85	95
8/26	82	100	75	90	100	95
8/27	80	83	65	88	92	78
8/28	85	80	73	97	88	92
8/29	62	53	87	80	92	97
8/30	97	57	87			
8/31				87	85	82
9/6	75	47	15			
Check	0	0	0	0	0	0

Table 25. Average number of dead larvae collected on drop cloths under Sevin treated trees over a 6 day period following treatment.

Treatment	Check
14	4

Table 26. Average grams of gypsy moth frass collected on drop cloths under trees treated with Sevin XLR on July 25, 1984.

	Average grams of frass			
Date:	7/25	7/27	7/30	8/1
Treatment	0.63	0.026	0.065	0.035
Check	2.93	6.86	6.01	0.86

Table 27. Average number of dead larvae collected on drop cloths under Sevin treated trees over a 10 day period following treatment.

Treatment	Check
56	0

Table 28. Average grams of gypsy moth frass collected on drop cloths under trees treated with Sevin XLR on August 6, 1984.

	Average grams of frass			
Date:	8/6	8/8	8/10	8/14
Treatment	10.965	0.173	0.0816	0.0283
Check	5.543	1.926	1.416	2.006

Table 29. Percent mortality of gypsy moth larvae fed foliage treated with Sevin XLR on August 6, 1984.

	Percent mortality								
	After 24 hrs			After 48 hrs			After 72 hrs		
Bioassay	INSTAR			INSTAR			INSTAR		
date	III	IV	V	III	IV	V	III	IV	V
8/6	100	100	100						
8/8	90	87	93						
8/10							93	93	86
8/13	75	72	78	88	72	78			
8/14	82	50	58	83	68	87			
8/15	77	52	68	82	70	77			
8/16	57	63	60						
8/17							77	53	62
8/20	52	73	48	72	77	70			
8/21	50	27	47	65	52	68			
8/22	63	48	62	72	58	78			
8/23	47	63	63	67	65	78			
8/24	73	62	73	73	77	75			
8/27	53	68	45	53	68	47			
8/28	63	57	45	67	65	55			
8/29	58	35	43	58	50	50			
8/30	27	3	25						
8/31				60	55	40			
9/4				67	49	25			
9/6	50	45	32						
Check	0	0	0	0	0	0	0	0	0

The development of the F-1 sterile male program for gypsy moth will require the release of dehaired eggs from an aircraft over the target site. It may be desirable to have released eggs stick to the sides of tree trunks and branches. It would therefore require mixing eggs into a slurry containing sticker and then dispersing them out of the aircraft or spraying eggs with a sticker as they leave the aircraft. A number of stickers were screened in an attempt to identify a suitable one for use. A suitable sticker has to stick the egg to the tree bark but, more importantly, not interfere with hatch and emergence of the neonate.

One-hundred dehaired eggs were submerged in each of a number of stickers for a period of one hour. Eggs coated with sticker were removed and placed in plastic petri dishes. Half were then rinsed with water and the remainder were left unrinsed. Eggs were incubated for one week at 26°C and percent hatch recorded. Stickers were tested at 4 concentrations on eggs that were held in cold storage for 158 and 173 days. Eggs held for this period of time normally start hatching within 48 hours.

Table 30. Percent hatch of 173 day chilled dehaired gypsy moth eggs 7 days after being dipped in various concentrations of stickers.

	Percent hatch and emergence							
	Concentration of sticker							
	100%		75%		50%		25%	
Sticker	NR	R	NR	R	NR	R	NR	R
RA-1990	0	72	50	85	55	65	66	79
TS-30	32	58	33	98	47	78	79	70
TS-85	13	58	19	86	42	87	67	74
TS-100	18	68	51	87	53	80	64	78
Rhoplex B-15	41	74	15	98	71	79	70	75
Rhoplex B-60A	1	57	29	80	66	78	67	71
NuFilm 17	0	0	0	0	0	26	0	10
Plyac	0	37	8	55	24	61	49	75
Bond	15	52	22	84	9	76	20	79
Chevron	0	11	0	94	15	90	59	70
Vaporgard	0	0	0	36	0	65	0	1
Exhalt-800	0	0	0	0	0	0	0	0
Sup-R-Stick	0	29	0	70	0	9	26	85
Gelva-2424	0	21	49	79	63	60	77	80
Acrylocoat	26	46	14	90	40	90	60	79
Triton B-1956	0	0	0	7	0	6	0	74
Mobait	52	61	26	93	63	81	53	71
Molasses							66	78
Bivert							43	73
CIB							35	70
Check	84	85	97	92	76	78	75	75

NR - Eggs were not rinsed after exposure to sticker
R - Eggs were rinsed with water after exposure to sticker

Table 31. Percent hatch of 158 day chilled dehaired gypsy moth eggs 7 days after being dipped in various concentrations of stickers.

	Percent hatch and emergence							
	Concentration of sticker							
	100%		75%		50%		25%	
Sticker	NR	R	NR	R	NR	R	NR	R
RA-1990	0	59	17	61	38	67	64	71
TS-30	18	30	21	73	66	61	76	68
TS-85	3	44	27	70	27	57	57	78
TS-100	7	51	14	71	37	71	60	65
Rhoplex B-15	21	51	64	67	75	72	65	68
Rhoplex B-60A	0	43	14	70	44	60	51	70
NuFilm 17	0	0	0	3	0	48	0	29
Plyac	0	17	0	50	3	54	35	60
Bond	1	39	15	71	16	57	30	72
Chevron	0	0	0	45	10	64	39	59
Vaporgard	0	0	0	44	0	46	0	3
Exhalt-800	0	0	0	0	0	0	0	0
Sup-R-Stik	0	17	0	16	1	51	25	70
Gelva-2424	0	26	22	26	22	51	63	68
Acrylocoat	23	58	15	70	4	80	67	59
Triton B-1956	0	0	0	6	0	4	0	54
Mobait	8	41	26	59	25	46	48	76
Molasses							53	66
Bivert							28	55
CIB							52	72
Check	64	51	61	71	76	72	78	76

NR - Eggs were not rinsed after exposure to sticker
R - Eggs were rinsed with water after exposure to sticker

An Auger system for dispersing dehaired gypsy moth eggs by aircraft was tested in the laboratory. The auger system was installed on a piece of plywood on top of a laboratory work bench. After checking a number of materials, we found that poppy seed was as close to actual gypsy moth egg mass size and density as anything readily available. Therefore, we purchased 24 pounds of poppy seed (@ $1.00/lb) at a local grain store. All calibration work was done with poppy seed. (Weight per 20 ml: poppy seed - 14.29 gm; gypsy moth viable dehaired eggs - 13.16 gm).

By weighing 1 ounce of dehaired eggs we found that this amounted to approximately 53 ml, by volume. Not knowing exactly how many ounces of eggs would be dispersed over the Maryland release plot we calibrated the equipment to deliver various amounts from 5 - 25 ounces per acre. By using 53 ml per ounce we needed the following amounts per acre:

5 oz/acre = 265 ml
10 oz/acre - 530 ml
15 oz/acre - 795 ml
20 oz/acre - 1060 ml
25 oz/acre - 1325 ml
30 oz/acre - 1590 ml

The Cessna Ag-Truck aircraft was set up for a 75' swath at 115 MPH. This means that the aircraft will cover 17.4 acres per minute. Therefore, in order to apply 5 ounces of dehaired eggs over a given acre the auger would have to deliver 4,611 ml of eggs per minute.

We first set the equipment up to deliver 5 ounces of eggs (265 ml) per acre. We made a number of runs and adjusted auger speed until we were as close to needed delivery as the equipment would allow. We then made 3 runs at the desired setting and averaged the readings. The equipment was calibrated for 5 dosages.

Table 32. Calibration of aircraft auger system and setting to deliver a given number of dehaired gypsy moth eggs.

Ounces eggs / acre	Zero max. setting	Eggs needed per unit 1/ ml	Actual egg output per unit ml
5	13	2,305	2,340
10	21	4,611	4,667
15	27	3,458 3/	3,450
20	32.5	4,611 3/	4,650
25	40 2/	5,764 3/	5,567

1/ When used in the aircraft there will be 2 auger units in operation.

2/ Maximum setting, a higher dosage would require treating the target area 2 times.

3/ Calibration based on 30 seconds, all others 1 minute.

The auger system will be located in the bottom of the insecticide hopper on the Cessna Ag-Truck aircraft. Discharged eggs will flow through tubes mounted under the wings and will escape through 3 openings on each side of the aircraft. In order to keep the auger system primed, it will require approximately 1.5 gallons of eggs. If possible, the system should be used with eggs only. If need be, poppy seed can be used as a carrier.

Viable eggs were passed through the system a number of times and hatch was not affected. Eggs were also passed through a vacuum cleaner without affecting hatch.

Table 33. Percent hatch of dehaired gypsy moth eggs that were passed through an auger system, shaker and vacuum cleaner.

Treatment	Times passed through system	Percent hatch of dehaired eggs
Vacuum cleaner	1	80 - 90
Lab shaker	1 hr.	80 - 90
"	2 hrs.	80 - 90
Auger system	1	80 - 90
"	5	80 - 90
"	10	80 - 90
Check	-	80 - 90

In Pennsylvania, dehaired eggs were dropped from a fire tower (75') and from the Cessna Ag-Truck aircraft (100') onto a hard surface. Resulting hatch of dropped eggs was 80 - 90 percent.

Once the equipment is installed in the aircraft some calibration adjustment may be needed.

In general, the system performed very well and should be usable for our 1985 dehaired egg drop.

For the past eight years 3 sticking agents have been used with Hercon formulations of gypsy moth pheromone when applied as a mating disruption material. RA-1645 was first used, followed by RA-1990 and then Phero-Tac 26. Although RA-1990 was believed to be the most effective sticker, the change to Phero-Tac 26 was made because of its registration for use over food crops. At this time RA-1645 and RA-1990 do not have this type of registration.

For maximum effectiveness, pheromone flakes should be applied with a good sticker so that individual flakes will stick to the foliage for at least 3 weeks. During our 1983 treatments using Phero-Tac 26, it was observed that most flakes were removed from the foliage within a 3 day period following treatment. This loss of flakes was due mainly to wind and rainfall.

In the hope of identifying a more suitable sticker, laboratory tests were conducted at the USDA, Otis Methods Development Center during February of 1984. Ms. Laura Zeoli of Hercon spent a number of days at the Otis facility assisting in the testing of a number of materials. Major emphasis was placed on testing materials that presently have registration for use over food crops. However, a number of unregistered materials were also tested.

Individual flakes (1/32 x 3/32 inch) were hand treated with sticker using a small paint brush for application. Some flakes were exposed to light amounts of sticker while others were treated with heavy amounts. Treated flakes were placed by hand onto foliage of tender northern red oak seedlings. The sticker was then allowed to dry for various amounts of time before being exposed to wind and rainfall. In most cases, 30 flakes were placed onto a seedling and this was replicated 3 times with each treatment. After each rainfall and/or wind treatment, all flakes remaining on the foliage were counted and percent loss computed. Rainfall was applied over a 4 week period.

Table 34. Percent loss of Hercon gypsy moth pheromone flakes, treated with various stickers, from oak foliage after 1 hour of drying time before exposure to wind and rainfall.

Sticker	Sticker amount	Percent loss after exposure to 1.0" rain	wind	1.5" rain	2.0" rain	wind	2.5" rain	3.0" rain	wind
RA-1990	Light	0	0	0	0	0	3	3	3
RA-1990	Heavy	0	0	0	0	0	0	0	0
Phero-Tac 26	Light	30	43	47	60	63	77	77	77
Phero-Tac 26	Heavy	20	30	40	47	60	73	80	80
75% RA-1990/ 25% Phero-Tac 26	Light	0	0	0	0	0	0	0	0
75% RA-1990/ 25% Phero-Tac 26	Heavy	0	0	0	0	0	0	0	0
50% RA-1990/ 50% Phero-Tac 26	Light	0	13	7	7	7	7	7	7
50% RA-1990/ 50% Phero-Tac 26	Heavy	0	7	7	7	7	7	7	7
Bond	Light	30	43	43	43	43	43	43	43
Bond	Heavy	3	13	7	17	17	17	17	17

Seedlings were exposed to 8 mph winds for 5 minutes.

Table 35. Percent loss of Hercon gypsy moth pheromone flakes, treated with various stickers, from oak foliage after 4 hours of drying time before exposure to wind and rainfall.

Sticker	Sticker amount	Percent loss after exposure to 1.0" rain	wind	1.5" rain	2.0" rain	wind	2.5" rain	3.0" rain	wind
RA-1990	Light	0	0	0	0	0	0	0	0
RA-1990	Heavy	0	0	0	0	0	0	0	0
Phero-Tac 26	Light	0	9	10	20	20	30	40	40
Phero-Tac 26	Heavy	1	10	10	13	13	20	27	30
75% RA-1990/ 25% Phero-Tac 26	Light	0	0	0	0	0	0	0	0
75% RA-1990/ 25% Phero-Tac 26	Heavy	0	3	3	3	3	3	3	3
50% RA-1990/ 50% Phero-Tac 26	Light	0	0	0	0	0	3	3	3
50% RA-1990/ 50% Phero-Tac 26	Heavy	0	3	3	3	3	3	3	3

Seedlings were exposed to 8 mph winds for 5 minutes.

Table 36. Percent loss of Hercon gypsy moth pheromone flakes, treated with various stickers, from oak foliage after 16 hours of drying time before exposure to rainfall.

Sticker	Sticker amount	Percent loss after exposure to 1.0" rain	2.0" rain	3.0" rain	4.0" rain	5.0" rain	7.0" rain
RA-1990	Light	1	1	1	2	3	3
RA-1990	Heavy	0	0	0	0	0	0
Phero-Tac 26	Light	38	71	87	96	100	100
Phero-Tac 26	Heavy	33	73	83	83	100	100
75% RA-1990/ 25% Phero-Tac 26	Light	1	1	2	2	2	2
75% RA-1990/ 25% Phero-Tac 26	Heavy	0	0	0	0	0	10
50% RA-1990/ 50% Phero-Tac 26	Light	4	4	6	6	6	6
50% RA-1990/ 50% Phero-Tac 26	Heavy	0	0	0	0	0	0
Plyac	Light	97	100	100	100	100	100
Plyac	Heavy	100	100	100	100	100	100
Bond	Light	12	23	27	30	30	31
Bond	Heavy	23	27	30	30	30	82

Table 37. Percent loss of Hercon gypsy moth pheromone flakes, treated with various stickers, from oak foliage after 16 hours of drying time before exposure to rainfall.

Sticker	Sticker amount	Percent loss after exposure to 0.25" rain	0.5" rain	1.0" rain	2.0" rain	4.0" rain
B 60A	Light	0	0	0	0	10
	Heavy	0	0	0	0	0
Exhalt-800	Light	17	43	90	100	100
	Heavy	0	0	20	80	100
NuFilm-17	Light	7	47	70	95	100
	Heavy	0	40	90	100	100
Spread-Zit	Light	0	0	0	13	82
	Heavy	0	0	20	30	100
Super-stik	Light	7	90	97	100	100
	Heavy	0	0	20	30	100
Target NL	Light	73	100	100	100	100
	Heavy	0	40	100	100	100
TS-30	Light	0	0	0	40	
	Heavy	0	0	0	40	
TS-85	Light	0	0	20	70	
	Heavy	0	0	0	30	
TS-100	Light	0	0	0	5	20
	Heavy	0	0	0	0	0
Vaporgard	Light	0	7	73	97	
	Heavy	0	0	0	30	
Polyco-2142	Light	25	60	100		
	Heavy	10	70	80		

Limited tests were also conducted with larger flakes (1/8 in.sq.). RA-1990 continued to be effective and the standard Phero-Tac 26 gave poor results.

Table 38. Percent loss of large Hercon gypsy moth pheromone flakes, treated with various stickers, from oak foliage after 16 hours of drying time before exposure to rainfall.

Sticker	Percent loss after exposure to 0.25" rain	0.5" rain	1.0" rain	2.0" rain	4.0" rain
Phero-Tac 26	6	43	80	99	100
RA-1990	1	1	1	1	1
25% Phero-Tac 26/ 75% RA-1990	0	0	0	1	1
50% Phero-Tac 26/ 50% RA-1990	1	1	1	1	3

RA-1990 was the most effective sticker tested followed by combinations of Phero-Tac 26 and RA-1990. Phero-Tac 26, the material presently being used, gave poor results when used alone. RA-1990 has also been the most effective sticker tested with Bacillus thuringiensis.

In areas where no food crops are affected, RA-1990 should be used with the Hercon formulation of gypsy moth pheromone. Testing should be conducted with RA-1990 and the new dispersal system before going operational. However, if pheromone is to be used in North Carolina during the 1984 season, it is recommended that RA-1990 be used.

An effort should be made by Hercon, Abbott, Zoecon and the U. S. Department of Agriculture to acquire clearance of RA-1990 for use over food crops. This has been started through talks with Monsanto's registration personnel. Because Monsanto will only furnish the material in bulk, Agway and Hercon have demonstrated an interest in marketing the material as an agricultural sticker.

Studies were conducted to test the attractiveness of female gypsy moth silhouettes, flat and embossed, to native male moths. Silhouettes were placed on trees and observed over a period of time. Some were treated on the back side with pheromone and others were not treated. Untreated ones attracted no male moths although a number of native adult males were flying in the general area. Silhouettes treated with pheromone did attract adult males, however, they gave no indication that they were aware of the artificial female moth. They did not appear to be attracted by visual sight. There was no attempt at mating by the native male moths, even when they came in contact with the artificial female.

Project Number: GM 3.1.1
Project Title: Field Studies with Bacillus thuringiensis
Report Period: October 1, 1983 - September 30, 1984
Report Type: Final
Project Leaders: W. H. McLane, F. A. Finney and T. Roland

A new strain of Bacillus thuringiensis (NRD-12) was compared to the standard strain (HD-1). Laboratory tests have demonstrated NRD-12 approximately 20 percent more effective than HD-1.

Table 1. Percent mortality of laboratory reared 2nd instar gypsy moth larvae 4 days after exposure to oak seedlings treated with 2 strains of BT at 2 BIU/96 oz/acre.

Material	Percent Mortality
SAN-415 (NRD-12) Zoecon	63
Thuricide 32LV	41
Thuricide 48LV	55
ABG-6163 (NRD-12) Abbott	44
Dipel 4L	18
Check	0

Using Dipel 8L as a spray material, micronair atomizers were compared with standard flat fan spray nozzles. Micronair atomizers have the capability to produce very fine droplets in a narrow size spectrum. Flat fan nozzles that have been widely used for the past number of years tend to produce larger droplets with a wide size spectrum.

Neat applications of Dipel 8L and Thuricide 48LV were sprayed from micronair atomizers and compared with treatments of 96 ounces per acre using micronair atomizers and flat fan nozzles.

Thuricide 48LV was tested against 3 gypsy moth instars using 16 BIU/96 oz/acre. Multiple applications at 5 day intervals were also tested with 16 BIU/96 oz/acre.

Table 2. Bacillus thuringiensis applications made on 50 acre woodland plots.

Material	Dosage/rate BIU/96 oz/acre	Nozzle type	Larval instar	Number applications
San-415	6	Flat fan	II	II
San-415	12	"	"	"
Thuricide 32LV	6	"	"	"
Thuricide 32LV	12	"	"	"
ABG-6163	6	"	"	"
ABG-6163	12	"	"	"
Dipel 6L	6	"	"	"
Dipel 6L	12	"	"	"
Dipel 8L	12 1/	Micronair	"	"
Thuricide 48LV	12 2/	"	"	"
Dipel 8L	12	"	"	"
Dipel 8L	12	Flat fan	"	"
Thuricide 48LV	16	"	II III IV	"
Thuricide 48LV	16	"	II III IV	II III IV

1/ Applied neat at 24 ounces per acre
2/ Applied neat at 32 ounces per acre

All BT mixes contained 2 percent RA-1990 sticker by volume. The sticker was screened through a 50-mesh screen as it entered the nurse tank.

Treatment plots were established in Tiadaghton Forest District near Woolrich, Pennsylvania. Using a compass, topographical maps, rope and surveyor tape, plots were established and allocated to the treatments listed in Table 3. Boundary lines were surveyed and marked in fluorescnt orange tape and each corner tree was marked with double fluorescent orange tape and a tag identifying corner and plot number. Minimum distance between plots was 400 feet. Plots were located so that there would be the maximum number of corners on or near roadways.

Treatment evaluation consisted of pre and post egg mass counts; egg hatchability tests, post spray larval counts and defoliation observations.

Within the center 10 acres of each plot, 20 prism points were established, 5 points on 4 parallel lines. During March and early April, pre-spray egg mass counts were made at each prism point. New egg masses were counted and recorded on each prism tree and within each fixed radius plot. Prism tree DBH was also recorded. A limited number of egg masses were collected from the field and returned to the laboratory for hatchability tests. Hatch was uniform at 80-90 percent.

Starting points for 5 minute larval counts were marked in each plot related to the NRD-12 study.

Table 3. Gypsy moth larval size at time of treatment.

Formulation	Dosage/rate BIU/oz/acre	Planned larval size	Instar at treatment I	II	III	IV	V
Dipel 8L 1/	12/24	II	5	95			
Dipel 8L 1/	12/96	II	5	95			
Dipel 8L	12/96	II	5	95			
Thuricide 48LV 1/	12/32	II	11	85	4		
NRD-12 (Zoecon)	6/96	II	11	85	4		
	12/96	II	11	85	4		
NRD-12 (Abbott)	6/96	II	0	61	39		
NRD-12 (Abbott)	12/96	II	0	61	39		
Thuricide 32LV	6/96	II	11	85	4		
Thuricide 32LV	12/96	II	5	65	30		
Dipel 6L	6/96	II	0	24	66	10	
Dipel 6L	12/96	II	0	24	66	10	
Thuricide 48LV	16/96	II	0	14	71	15	
Thuricide 48LV	16/96	III	0	0	61	38	1
Thuricide 48LV	16/96	IV	0	0	7	80	13
Thuricide 48LV 2/	16/96	II	0	0	40	59	1
	16/96	III	0	0	7	80	13
		IV	0	0	20	80	0

1/ Eight mini-micronair nozzles were used
2/ Multiple applications made to the same plots

Applications were made with a Cessna Ag-truck aircraft. Flat fan spray nozzles were used for treatments not utilizing micronair atomizers. Nozzles were pointing 45° into the slip strem and were supplied with 38-40 PSI. The aircraft was equipped with a 50-mesh in-line screen and quick drain valves. All nozzles were equipped with 50-mesh screen. The material was applied at 120 mph in a 70 foot swath width 10 feet above the tree tops.

All mixing was conducted in a nurse tank and then material was pumped into the aircraft. Water was first added to the nurse tank, then BT and sticker. Mixes were blended by agitation for at least 10 minutes before being loaded into the aircraft. The nurse tanks was equipped with a 50-mesh in-line screen.

Following treatment, a series of 5 minute larval counts were made in NRD-12 plots and all other plots associated with the NRD-12 application. Three parallel lines were walked within the center 10 acres of each plot and larvae counted on each line for a period of 5 minutes. An average of the 3 counts was then computed. Table 4 gives percent change between an average reading taken 7 days post treatment and one taken 21 days following treatment. Populations seemed to increase because the larger larvae found at 21 days were more apparent, thus, rendering higher counts.

Table 4. Percent change in larval numbers between first and last 5 minute counts made in treated and untreated plots.

Formulation	Dosage BIU/acre	Percent change
NRD-12 (Zoecon)	6	+ 817
NRD-12 (Abbott)	6	+ 127
Thuricide 32LV	6	+ 317
Dipel 6L	6	+ 42
NRD-12 (Zoecon)	12	+ 559
NRD-12 (Abbott)	12	- 8
Thuricide 32LV	12	+ 104
Dipel 6L	12	+ 10
Check	-	+ 102

Post-spray defoliation surveys were made in all spray plots and checks at the time of peak defoliation in mid-July. Treated areas and large acreage of untreated forest land had far less defoliation than expected. Within all treated plots, defoliation was so light (0 - 5 percent) that it was impossible to distinguish differences between plots. Check plots averaged slightly higher defoliation (15 percent). All plots were also observed and photographed by air. Upon observing limited defoliation and in an attempt to quantify differences among treatments, all plots were given a 0 - 10 rating based on the number of large larvae and pupae observed. The more larvae observed, the higher the number.

Table 5. Average rating from 0 - 10 for each treatment based on number of large larvae and pupae observed at peak defoliation time.

Treatment	Dosage BIU	Rate oz.	Rating 0 - 10
Dipel 8L 1/	12	24	5
Dipel 8L 1/	12	96	3
Thuricide 48LV 1/	12	32	7
Dipel 8L	12	96	5
NRD-12 (Zoecon)	6	96	6
NRD-12 (Zoecon)	12	96	6
Thuricide 32LV	6	96	5
Thuricide 32LV	12	96	3
NRD-12 (Abbott)	6	96	4
NRD-12 (Abbott)	12	96	5
Dipel 6L	6	96	4
Dipel 6L	12	96	3
Thuricide 48LV	16	96	3
Thuricide 48LV 2/	16	96	2
Thuricide 48LV 3/	16	96	3
Thuricide 48LV 4/	16	96	0
Thuricide 48LV 5/	16	96	0
Thuricide 48LV 6/	16	96	0
Check	-	-	5

1/ Eight mini-micronair atomizers used. All other treatments with 8003 flat fan nozzles
2/ Applied to 3rd instar larvae
3/ Applied to 4th instar larvae
4/ 2 applications 5 days apart
5/ 3 applications 5 days apart
6/ 4 applications 5 days apart

Following fall foliage drop, post-spray egg mass counts were made in each plot. Twenty points were sampled in each plot using the same technique as was used for pre-spray calculations. All ribbon and tags were removed from each plot.

Table 6. Percent egg mass change in BT plots treated with 96 ounces per acre using flat fan spray nozzles unless otherwise stated.

Formulation	Dosage BIU/ac	Pre-spray em/acre	Post-spray em/acre	Percent change
Dipel 8L 1/	12	504	62	- 88
Dipel 8L 2/	12	754	46	- 94
Thuricide 48LV 3/	12	319	128	- 60
Dipel 8L	12	252	32	- 87
NRD-12 (Zoecon)	6	513	97	- 81
NRD-12 (Zoecon)	12	611	34	- 94
Thuricide 32LV	6	409	75	- 82
Thuricide 32LV	12	241	20	- 92
NRD-12 (Abbott)	6	368	65	- 82
NRD-12 (Abbott)	12	442	54	- 88
Dipel 6L	6	304	55	- 82
Dipel 6L	12	280	47	- 83
Check 4/		268	111	- 59
Thuricide 48LV	16	472	20	- 96
Thuricide 48LV 5/	16	306	55	- 82
Thuricide 48LV 6/	16	247	33	- 87
Thuricide 48LV 7/	16	466	64	- 86
Thuricide 48LV 8/	16	2,412	20	- 99
Thuricide 48LV 9/	16	1,598	5	- 99
Check	-	121	33	- 73

1/ Eight mini-micronair atomizers used to apply 24 ounces per acre undiluted.
2/ Eight mini-micronair atomizers used to apply 96 ounces per acre
3/ Eight mini-micronair atomizers used to apply 32 ounces per acre undiluted.
4/ Check for above treatments only
5/ Applied to 3rd instar larvae
6/ Applied to 4th instar larvae
7/ Two applications 5 days apart
8/ Three applications 5 days apart
9/ Four applications 5 days apart

Five minute larval counts were erratic and not dependable. This was caused by doing the same plots at different time of the day under varying weather conditions. Counts were also influenced by the increased movement of later instar larvae to the lower position of the tree, increasing total numbers to count. We were, therefore, unable to use this evaluation to analyze and compare treatments.

Defoliation was so light that no significant difference could be detected between treatments. Based on size and number of egg masses per acre it was anticipated that moderate defoliation would occur.

Based on a (0-10) rating, Dipel 8L applied neat using micronair atomizers was as effective as diluted applications at 96 ounces per acre using flat fan nozzles. When a Dipel 8L neat application was compared with a 96 ounce per acre diluted using micronair atomizers, the diluted application was more effective. Thuricide 48LV applied by micronair atomizers gave poor results when used undiluted. When Dipel 8L was applied at 96 ounces per acre with both atomizers and nozzles the micronair atomizer treatment was most effective. There was little difference between treatment of the HD-1 and NRD-12 strains of BT. If anything, the HD-1 strain appeared to be slightly more effective in the field. However, based on the mortality that occurred in the check, there is no sound basis to say one strain is more efficacious than another. Applications of Thuricide 48LV at 16 BIU/96 oz/acre were effective in controlling II, III and IV instar gypsy moth larvae. Multiple applications of Thuricide 48LV using 16 BIU/96 oz/acre were extremely effective in controlling gypsy moth larvae.

Pre and post spray egg mass count data thoroughly support the findings of the (0-10) plot rating.

Summary

Final results would have been more meaningful if we had not experienced a population collapse in all check plots. This again demonstrates the lack of techniques to fully predict developments within a given gypsy moth population.

We were not able to distinguish a statistical difference between the HD-1 and NRD-12 strains. However, based on check mortality and the fact that laboratory data continue to support NRD-12 as being more efficacious than HD-1, I feel that field work should continue during 1985. I would recommend testing only one dosage and rate, preferably 6 BIU/96 ounces/acre.

Micronair atomizers were found to be effective in applying neat and diluted applications of BT. They appeared to be more effective than flat fan nozzles when making applications of 96 ounces per acre. If we are to master the use of neat applications of BT for gypsy moth control (quart or less per acre) work should continue in this area using micronair atomizers.

We found that applications of 16 BIU/96 ounces/acre can be effective in controlling larger instar gypsy moth larvae.

It was also demonstrated that multiple applications of Thuricide 48LV can be extremely effective against light to medium density populations of gypsy moth.

All formulations handled very well and no mixing problems were encountered. In line and nozzle screens were regularly cleaned and no clogged nozzles were encountered throughout the spray program.

It would be adviseable for Zoecon to use the standard metal 55 gallon drum. During 1984 our Thuricide was received in 55 gallon blue drums that had little if any lip on them. They were very hard to handle and we experienced an excessive buildup of pressure in some containers.

Project Number: GM 3.1.2
Project Title: 1983 Eradication Projects with Bacillus thuringiensis
Report Period: October 1, 1983 - September 30, 1984
Report Type: Interim
Project Leaders: W.H. McLane, G. Moorehead, K. Kruse and D. Keim

This project is ongoing and additional information will appear in the next Otis Methods Development Center Progress Report.

Project Number: GM 8.2.2
Project Title: Radiological Sterilization of Male Gypsy Moths
Report Period: October 1, 1983 - September 30, 1984
Report Type: Interim
Project Leader: V.C. Mastro

This project is ongoing and additional information will appear in the next Otis Methods Development Center Progress Report.

Project Number: GM 0.2.7
Project Title: Sterile Male Trial, Berrien County, Michigan
Report Period: October 1, 1983 - September 30, 1984
Report Type: Final
Project Leader: V. C. Mastro

Results of trapping (32 trap/sq.mi.) in 1984 were again negative (i.e., no males were trapped). This is the second consecutive year of negative trap results and we believe eradication has been achieved. Therefore, we will terminate any further activities at this site. The following table is a brief summary of the whole program.

A Pilot Study for Demonstrating the Sterile Male Technique

Detection, Delimitation and Management of an Isolated Sparse Gypsy Moth Population in Hagar Shores, Michigan.

Year	Activity	Monitoring Population		
		Stage	Technique	Sample Size
1978	Detection	Larva	---	–
		Adult(M)	1 Trap/mi^2	1
		Egg	Inspection	0
1979	Delimitation	Larva	---	–
		Adult(M)	32 Traps/mi^2	90
		Egg	Inspection	1
1980	Delimitation	Larva	Banding	110
	Intervention	Adult(M)	32 Traps/mi^2	274
		Egg	Inspection	47
1981	Delimitation	Larva	Banding	14
	Intervention	Adult(M)	32 Traps/mi^2	50
		Egg	Inspection	1
1982	Delimitation	Larva	Banding	0
	Intervention	Adult(M)	32 Traps/mi^2	1
		Egg	Inspection	0
1983/ 1984	Delimitation	Larva	---	–
		Adult(M)	32 Traps/mi^2	0
		Egg	---	–

Project Number: GM 1.2.1
Project Title: Behavior and Ecology of Immature Gypsy Moths: Population Quality and Competitiveness of F1 Sterile Individuals
Report Period: October 1, 1983 - September 30, 1984
Report Type: Final
Project Leaders: D. R. Lance, J. S. Elkinton, C. P. Schwalbe and V. C. Mastro

During outbreaks of the gypsy moth Lymantria dispar (L.), larvae remain in the canopy and feed on and off throughout the day and night. In low-level populations, late instar larvae feed nocturnally and spend the day resting in protected sites away from the foliage. Development is 1-3 weeks faster during outbreaks, but the resulting adults are smaller and less fecund compared to those in low-level populations. A series of studies was undertaken to determine environmental factors that effect these changes in gypsy moth larvae.

Initially, scaffolding and (when necessary) night vision equipment was used to make observations of larval behavior in high- and low-density populations. Aside from the difference in feeding rhythms, larval feeding and food-seeking behaviors in the field were found to be fairly similar regardless of population density; however, at outbreak sites, larvae fed in shorter bouts and switched feeding sites relatively frequently. In 24 h laboratory tests, field-collected fifth instars maintained feeding rhythms that were characteristic of their source populations.

In the laboratory, pupal weights declined when larvae were exposed to crowding, were partially starved, or were reared either on leaves from heavily defoliated sites or on artificial diets that contained tannic acid (TA). Starvation and TA also reduced development rates. Development was similar to that of control insects when larvae were reared on leaves from trees defoliated in the previous year, exposed to several other density-correlated stress treatments (exposure to large quantities of fresh silk, sublethal doses of NPV, or freeze-dried larvae), or field-collected (as eggs) from different-density populations.

The proportion of feeding that was done at night was significantly reduced when larvae were reared either on diet containing TA or on leaves from a heavily defoliated site. This did not occur in response to any other treatment, suggesting that defoliation-related declines in leaf quality cause the gypsy moth's density-related shift in feeding rhythms.

On sunny days in the field, body temperatures of late instar larvae were several degrees higher at outbreak sites than they were in low-level populations. In the laboratory, larvae developed 1-2 weeks faster when exposed to simulated "outbreak" temperature regimes rather than simulated "low-density" temperatures; temperature differences alone can account for the rapid development during outbreaks.

Initially, studies on F1 sterile larval competitiveness (e.g. egg hatch synchrony, larval survival and development, comparisons of behavior) were included in this section. These studies are continuing, but they have been transferred to other project titles (e.g. GM 3.2.5).

Project Number: GM 1.2.4
Project Title: Mass Trapping
Report Period: October 1, 1983 - September 30, 1984
Report Type: Interim
Project Leaders: C. P. Schwalbe, V. C. Mastro

In field tests performed in 1982 and 1983 with simulated populations of released adults, it was shown that male capture rates generally increase as trap density increases from 2.5 - 25 traps/ha. However, female mating success was not correlated with proportions of released males captured since at the 2 highest trap densities (7.5 and 25 traps/ha) capture rates were similar but mating success was markedly reduced in plots with 25 traps/ha. Time studies showed that as trap density increased, the mean elapsed time (hours after release) for male capture decreased and it was proposed that rapidity of male capture was an important factor defining the effectiveness of mass trapping in preventing mating. At lower trap densities, some males mate before they are captured in traps. In the 1984 experiments described here, we describe more accurately the hourly occurrence of mating and trap catch in trapped plots.

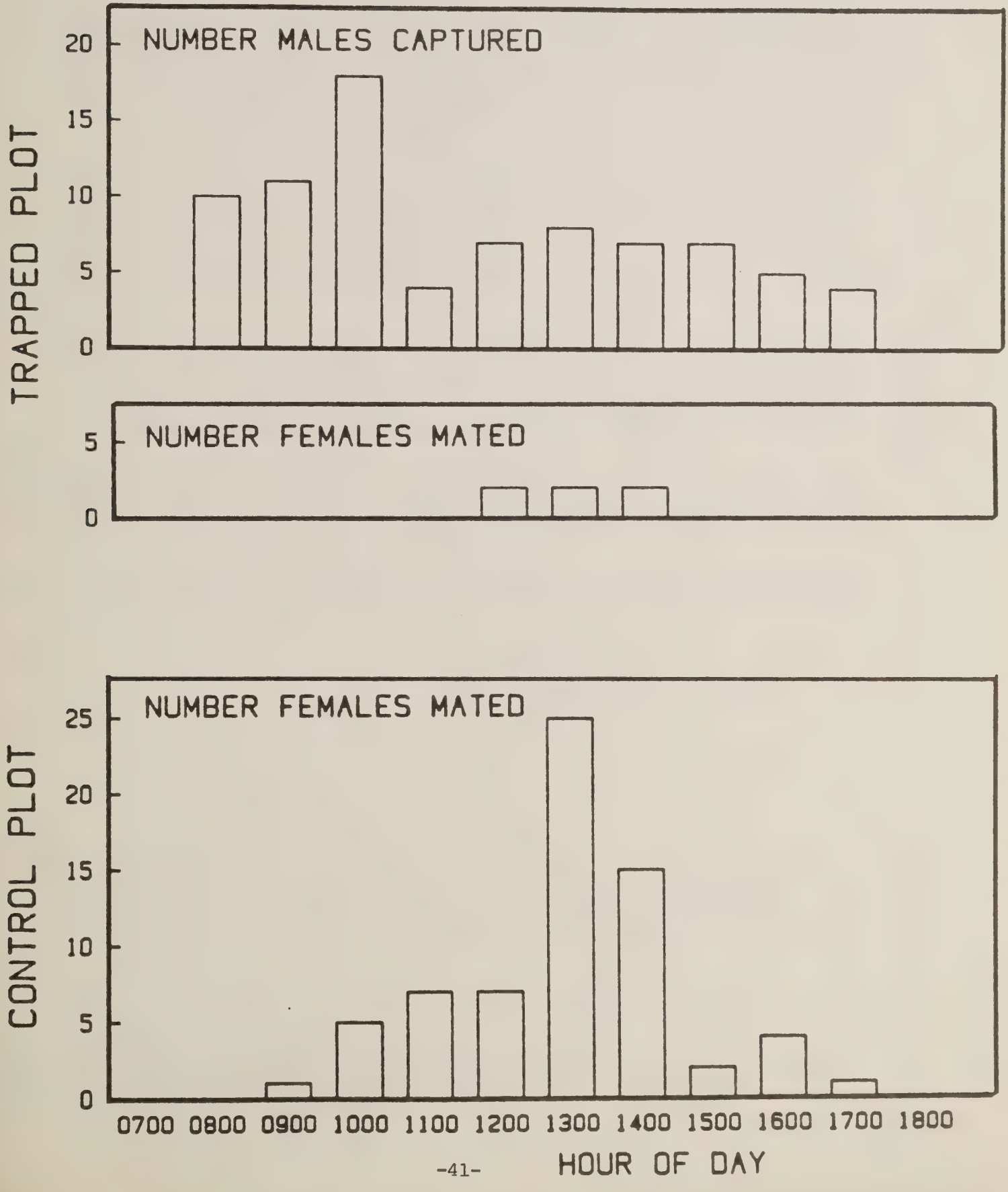
The studies were conducted in 9 ha plots that contained 25 traps per ha; all 225 traps were checked hourly during the experiments. Adults were released in the central 4 ha of each plot between 6:00 a.m. - 7:00 a.m. at the rate of 25 pairs per ha, so the starting ratio was 1 trap:1 pair. All females were checked every hour during the experiment and since copulation usually lasts at least 60 minutes, we stood a good chance of observing the mating of all females. Every time a male was observed mating a female, he was marked so that we could follow his activities later that day. Using colored dots on either or both wings, individual males could be identified as to the hour they mated.

The daily pattern of events in the Massachusetts tests (on the following chart) shows that female mating in the untrapped control plot occurred between 0900 and 1700 hours with peak activity around 1300 and 1400 hours. The small amount of mating in the trapped plot occurred between noon and 1400 hours. Male trap catch, on the other hand, tended to occur earlier in the day.

Maryland results (see following chart) show a similar trend with female mating in control plots tending toward afternoon with most males caught in traps prior to 1300 hours. Again, the small amount of mating in the trapped plot was observed during the same time segment as in the control plot.

The following tables summarize mating observations made in the trapped and control plots in Massachusetts and Maryland.

1984 MASS TRAPPING MASSACHUSETTS



1984 MASS TRAPPING MARYLAND

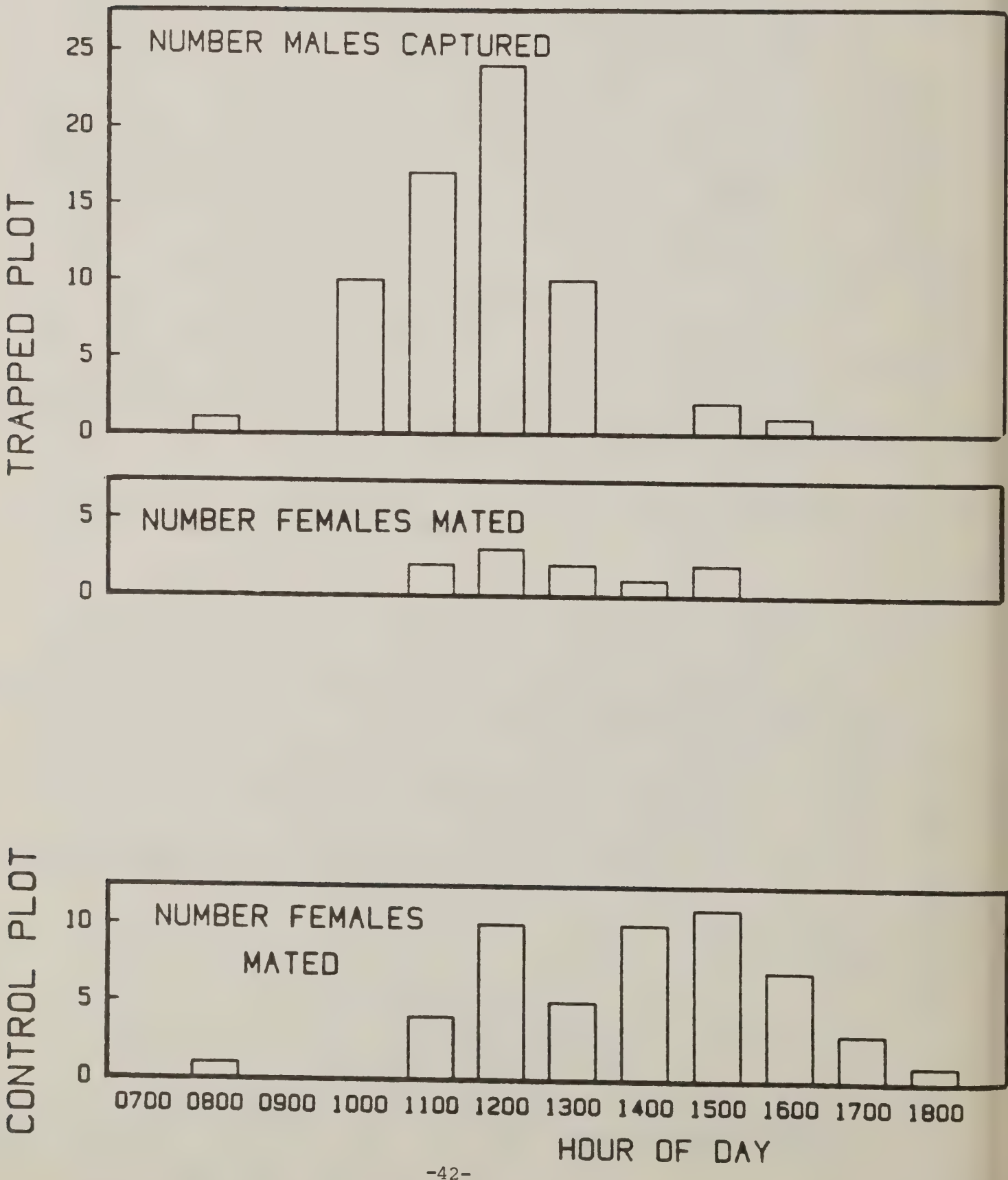


Table 1. Mating success and time to mate/capture in Massachusetts and Maryland mass trapping tests.

	Massachusetts		Maryland	
	Control	Trapped	Control	Trapped
Number females mated	67	6	51	10
Number females not mated	33	94	36	81
Number males trapped	-	81	-	65
Mean hours to mate	5.9	6.0	6.9	5.8
Mean hours to capture	-	4.6	-	4.7

In the Massachusetts tests, 67 of 100 females were mated in the control plots with only 6 of 100 mated in the trapped plot; 81 males (out of 100 released) were caught in traps. An important point is that in both the treated and control plots, the average time for females to be located and mated was 5.9 to 6.0 hours after release whereas the average male only took 4.6 hours to get caught in a trap.

Likewise in Maryland, 51 females mated in the control plot contrasted with only 10 in the trapped area; 65 males were caught in traps. Females spent 5.8 to 6.9 hours getting located and mated contrasted with an average of 4.7 hours for males to be captured in the traps. Clearly, in both locations, trap catch occurred before female mating.

Multiple mating by male gypsy moths has been previously documented in some laboratory tests and since we marked males individually when they were found mating, we were able to follow their subsequent mating activity. The following table summarizes those observations.

Table 2. Incidence of multiple mating by male gypsy moths in Massachusetts and Maryland mass trapping test plots.

	Massachusetts		Maryland	
	Control	Trapped	Control	Trapped
Number of males that mated:				
one time	24	6	14	8
two times	7	0	11	1
three times	7	0	5	0
four times	2	0	0	0
	40	6 [1]	30	9 [2]

1/ 5 males were subsequently captured; 1 was unaccounted for

2/ 5 males were captured in the hour after they last mated; 4 were unaccounted for.

The Massachusetts studies revealed that the 67 females that were mated in the control plot were mated by only 40 males. 24 males mated only once but the remaining 16 moths accounted for 43 matings; 2 males inseminated 4 females each. In the trapped plot only 6 males mated and those were all single matings. 5 of those males were subsequently captured in traps and 1 was unaccounted for.

In Maryland, the 51 matings in the control plot were done by only 30 males, 11 of which mated twice and 5 mated 3 females each. In the trapped plot, 8 males mated once and 1 mated twice. Five of those 9 mating males were caught in traps the hour after they last mated and 4 of the mating males were never seen again.

In both studies, multiple mating males accounted for most of the mating in the control plots, so minimizing the time available to males to multiple mate has a substantial impact on female mating success.

Project Number: GM 1.2.7
Project Title: Gypsy Moth Mating Disruption - Oconomowoc, Wisconsin
Report Period: October 1, 1983 - September 30, 1984
Report Type: Final
Project Leader: C. P. Schwalbe

The history of this infestation since 1977 was chronicled in the last Progress Report. The original objective was to eradicate the infestation through the mating disruption method. Actions since 1982 have been mainly mass trapping and a small section near Okauchee Lake of the original population appears to have been successfully eliminated by the trapping program (See 1982-83 Progress Report). The findings of the project are:

1) Mass trapping is associated with the gradual reduction and ultimate disappearance of a population near Okauchee Lake.

2) The combined effects of broadcast disparlure (Hercon flakes) and limited ground application of Sevin is associated with large parts of the original 1978 infestation being reduced to non-detectable levels. In 1978, 700 moths were captured and the infestation occupied 425 acres. 1984 delimitation survey revealed no evidence of infestation in that area thus treated.

3) A population (separate from the original infestation and in an area never treated) has been located and is reproducing.

Project Number: GM 2.2.4
Project Title: Induced Inherited Sterility Trial - Horry County, South Carolina
Report Period: October 1, 1983 - September 30, 1984
Report Type: Interim
Project Leaders: V. C. Mastro and T. M. ODell

The last annual report summarized the results of this trial to date. Because 1983 results indicated that a small remanent population may have survived, the "core" area and area around it (i.e. total of a square mile) was trapped at a rate of 32 traps/sq.mi. High-capacity milk carton traps were used for this survey. No males were trapped in 1984 indicating eradication has been achieved, however, the same area will be trapped at the same rate in 1985 as in 1984 to confirm eradication.

Project Number: GM 2.2.5
Project Title: Mass Trapping Pilot Study - Monona, Wisconsin
Report Period: October 1, 1983 - September 30, 1984
Report Type: Interim
Project Leaders: C. P. Schwalbe, V. C. Mastro

In 1981, a small, isolated gypsy moth infestation was located in Monona, Wisconsin. Because the infestation was adjacent to a lake (Monona) and in a residential area, it was resolved to attempt eradication through the use of mass trapping. Details of the history (with maps) are in 1982 and 1983 Progress Reports. It should be noted that the reduction in male density observed from 1981-82 was probably due to extremely cold 81-82 winter temperatures. The following is a summarization of data collected since the project began:

Table 1. Results of male moth trapping and larval and pupal sampling (under systematically placed burlap bands) in Monona, Wisconsin.

	1981	1982	1983	1984
Number male moths captured	236	113	40	7[5/]
Estimated male population	2360[1/]	151[2/]	53[2/]	8[6/]
Number larvae and pupae		31	34	0
Estimated ha infested		13	8	1
Estimated number of pairs (season-long)/ha		11.6[3/]	6.7[3/]	8.0[3/]
Flight period (days)		21	21	21
Estimated pairs/ha/day		0.6	0.3	0.38
Estimated number females mated		13.3[4/]	4.7[4/]	0.48[7/]

1/ Assuming 10% capture rate with 32 traps/mi^2
2/ Assuming 75% capture rate with 7.5 traps/ha
3/ Assuming 1:1 male:female sex ratio
4/ Number females mated/season = (no. pairs/ha/day)(.088)(flight period)(ha infested) where .088 = mating success of 2.5 females/ha in area containing 7.5 traps/ha.
5/ 25 traps/ha deployed in 1984
6/ Assuming 86% capture rate with 25 traps/ha
7/ Number females mated/season = (no. pairs/ha/day)(.06)(flight period)(ha infested) where .06 = mating success of 2.5 females/ha in area with 25 traps/ha

Since calculations indicate less than one female was mated in 1984, eradication of the population should have been achieved. 1985 trapping results will judge the accuracy of that prediction.

Project Number: GM 3.2.1
Project Title: Mating Disruption Demonstration Project - Carteret County, North Carolina
Report Period: October 1, 1983 - September 30, 1984
Report Type: Final
Project Leader: C. P. Schwalbe

This project (described in detail in the last Progress Report) was designed to use the mating disruption technique to demonstrate eradication of a small, isolated gypsy moth infestation located in coastal North Carolina (Carteret Co.). A brief summary of the project follows.

In 1982, 38 moths were captured in delimitation survey and 3 egg masses were found. The infestation was judged to occupy 150 acres. In 1983, 42 larvae and pupae were collected from 7 burlapped trees. The disruptant (Hercon flakes) was applied May 25, 1983 at 20 g ai/acre. During the period when adult flight was expected, virgin, sterile, female moths were placed throughout the area to monitor mating success. Out of 80 females collected, none was mated. Only one male moth was caught in a trap (near the site where egg masses were found in 1982.

We were encouraged by these findings, but, recalling a previous instance where such results were misleading, (pheromone-baited traps are ineffective in detecting moths and are of questionable value in estimating mating success in a disparlure sprayed area) we resolved to apply disparlure again in 1984 if burlap banding that spring showed that a population persisted. About 300 burlap bands placed in the area facilitated the collection of 78 immatures in 1984, indicating that significant reproduction had taken place in 1983 or a new introduction had occurred (which is not likely, based on the distribution of larvae). At that point, State officials chose to terminate the project (and the infestation) by multiple ground applications of Bacillus thuringiensis and dimilin. Fourteen moths were subsequently captured in delimitation survey indicating that approach was not completely effective.

Project Number: GM 3.2.2
Project Title: Partially-Sterile Male (F-1) Pilot Study, Kent Co., Maryland
Report Period: October 1, 1983 - September 30, 1984
Report Type: Interim
Project Leaders: V.C. Mastro, K. Tatum, T.M. O'Dell, and R. Webb

The test design used for this pilot study was fully described in the last annual report. In addition, preliminary data from the 1983 field season were reported. This report will provide a more complete analysis of the 1983 data, and give preliminary findings for 1984.

Burlap bands, for larval survey, were again placed in all four wood lots. Table 1 presents the results of the larval survey for 1984. Peak numbers of immature gypsy moths were found under burlap bands during the second observation period, which was approximately a week later than when peak occurred in 1983. A comparison of the numbers of immatures found at the peak observation period for both years is presented on Table 2. The number of immatures found under bands was higher in 1984 in all plots, however, the smallest rate of increase was in plot 2, or the plot which had been treated with partially sterile males (10 krad) in 1983.

Adult male trapping was conducted in the same manner in 1984 as it was the previous year. Traps on plots 1 and 4 (control plots) were checked on a weekly basis while traps on plot 2 (sterile male release plot) were checked daily. Results of trapping for both years are summarized on Table 3. In 1984, large numbers of males were trapped earlier in the season than expected based upon larval phenology. This early trap catch was not apparent in 1983. The earliest that pupae were found under burlap bands in any of the plots in 1984, was on June 19. If the pupal stage was 14 days, the earliest that any native males should have been trapped was on July 3, and then very few should have been trapped. In Table 4 we consider any males trapped on or before July 4, as migrant males, and subtract these from the plot totals. Although some of the males trapped after July 4, may have also been migrants, we are not able to identify and separate these, and simply considered them all native males. A larger proportion of the total catches were determined to be migrant males in Plots 1 and 2. These plots are relatively close together, and Plot 4 was located ca. 4 miles further south. In 1984, areas with defoliating gypsy moth populations occurred 8 miles to the east and to the west of the plots.

The male adult survey indicates that the population within the control plots increased in 1984 (i.e. number of males captured in Plot 1 nearly doubled, and in Plot 4 increased over thirteen times). Male trap captures in the treatment plot remained nearly constant, even though the 1983 larval and adult trapping data indicate that it contained the highest initial insect density.

Table 1. Results of 1984 burlap band survey, Kent co., Maryland - 1984.

Plot #	No. of burlap bands placed	Observation number and dates observed											
		1st obs. 6/12-15	2nd obs. 6/20-21		3rd obs. 6/25-28		4th obs. 7/2-6		5th obs. 7/9-12		6th obs. 7/16	
		no. larvae	no. larvae	no. pupae[1/]	no. larvae	no. pupae	no. larvae	no. pupae	no. larvae	no. pupae	no. larvae	no. pupae
3	210	671	687	20	287	44	38	64	3	21	1	2
1	360	21	35	1	26	29	12	39	3	41	0	2
4	1069	132[2/]	177[2/]	6	107	69	25	110	3	35	0	4
2[3/]	936	256	337	44	119	106	31	131	3	42	0	3

1/ Includes prepupae and pupae
2/ Observations for the first check of plot 4 made between (6/7-6/12) and for the second check between (6/15-6/19).
3/ Sterile Release

Table 2. Number of immatures found under burlap bands in 1983 and 1984.

Year	Observation Period	Number of immature (larvae & pupae) found under bands			
		Plot 1	Plot 2	Plot 3	Plot 4
1983	(6/14-6/16)	21	353	16	24
1984	(6/15-6/21)	36	381	707	183

Table 3. Number of males captured in high capacity (milk carton) traps in plots in Kent Co., Maryland.

Plot No.	No. of Traps	No. of Males Captured	
		1983	1984[1/]
1	18	665	1036 (1833)
4	35	229	3030 (3451)
2[2/]	35	2,104	2434 (4118)

1/ Numbers in parentheses are total numbers of males captured including migrant males - see text.

2/ Substerile males (10 krads) released in this plot in 1983.

Analysis of hatch data from feral egg masses (plugs collected at the end of the 1983 season) and from monitor females placed in plot in 1983, is now complete (Table 4). Both types of egg masses were evaluated by determining the percentage hatch of embryonated eggs and comparing it with the mean percentage hatch from two groups of control matings (i.e. A = untreated males x untreated females, and group B = males treated with 10 krads as 8-11 day-old pupae x untreated females). The test statistic used for this comparison follows:

$$C = D^2/S^2 - 3.841$$

where $D = \text{arcsine}\sqrt{\frac{\text{no. of eggs in the sample that hatched}}{\text{total no. of embryonated eggs in the sample}}} - \text{arcsine}\sqrt{\bar{x}}$ proportion of embryonated eggs which hatched in A or B.

S = Standard deviation of the mean proportion of eggs which hatch in A or B

3.841 = a chi square tabular value for one degree of freedom at the .05 probability level.

By testing the proportion hatch in an egg mass of unknown male parentage against hatch of both control groups (A or B), it was determined if the percentage hatch was significantly different than either group. For example, if testing an unknown egg mass against group A results in a calculated negative C, then the sample egg mass is not significantly different from the A population. In cases where the analysis resulted in hatch of the sample egg mass being not significantly different than either group, its parentage was considered unknown.

Table 4. Mating type determinations of egg masses deposited by feral females and laboratory females placed as monitors in plots in Kent Co., Maryland - 1983.

Plot No.	Female Type	No. of egg mass of each mating type		Sterile: Fertile Ratio	No. of Egg Masses of Unknown Parentage[1/]	No. of Egg Masses Not Evaluated[2/]
		Feral Male	Released (10 krad) Male			
2	monitor females	18	338	18.8:1	11	113
2	feral females	1	69	69:1	1	1
4	monitor females	9	1	1:9	0	111
4	feral females	0	1	0:1	0	0

1/ Egg masses with proportions of embryonated eggs which hatch that were not significantly different than either control group A or B.

2/ Egg masses which contained less than five embryonated eggs.

Also excluded from the analysis, were all egg masses which contained less than five embryonated eggs. It was felt that these should be excluded from the analysis because there was the possibility of mistaking a discolored unembryonated egg or a parasitized egg for embryonated eggs. In effect, this biases the estimate of the mating ratio against sterile matings (10 krad male). Occasionally, mating of 10 krad treated males x normal females results in egg masses with a very low proportion of the total eggs embryonated. A total of 480 monitor females which oviposited at least one egg, were recovered from Plot 2.

The evaluation of egg masses for mating type was carried through the F-1 generation. Where possible, progeny from egg masses were reared to the adult stage and mated to normal laboratory reared moths. When evaluated, these F-2 egg masses will provide a more concise determination of the mating type than the evaluation of the F-1 egg masses. These results will be presented in the next annual report.

Preliminary evaluation of both feral and monitor F-1 egg masses indicates that sterile to fertile overflooding ratios were high in the release plot. If these estimates of mating success of both types of males (feral and 10 krad released males) are accurate, there is a large difference between the egg masses from monitor females and those from feral females. A reasonable explanation is that feral females are only present during a small period of time compared to monitor females, which were placed daily over a 38-day period. Perhaps during the period feral females were present, sterile:feral male over flooding ratio were particularly favorable and hence the high mating ratio.

In 1983, the first feral pupa was found under a burlap band on June 15, and by the next check, (June 23-24) there were 32 pupae and 16 prepupae present. Assuming that the pupal period was 14 days, most feral females would have eclosed after July 4. Daily male-trap records indicate that feral male flight began to increase on June 30, but peaked between July 12 and July 20. The estimated sterile:feral mating ratio during this period for monitor females was 255S:1F, not nearly as high as the estimated mating ratio for fertile females. The information presented on Table 5 indicates a relationship between S:F mating ratio and S:F trap ratio. Even though the relationship appears to be imperfect, the estimates of S:F mating ratios are preliminary, and are in any case, based on only a small number of monitor females.

In 1984, larvae and pupae were collected from the release plot (plot number 2) and reared to the adult stage. To determine if there were F-1 progeny of released males, or progeny of feral males, they were mated as one day old adults with normal untreated laboratory insects. The results of evaluating these egg masses will be presented in the next annual report.

Project Number: GM 3.2.5
Project Title: Sterile Male Technique - Studies on the Feasibility of Releasing F-1 Sterile Gypsy Moths as Eggs
Report Period: October 1, 1983 - September 30, 1984
Report Type: Interim
Project Leaders: D. R. Lance, V. C. Mastro, C. P. Schwalbe and T. M. ODell

In many Lepidoptera, complete sterility occurs in otherwise viable F_1 individuals when male P_1 pupae are treated with a proper sub-sterilizing dose of gamma radiation. This phenomenon potentially allows the deployment of sterile insects as eggs: sub-sterile males can be outcrossed in the laboratory, and the resulting eggs (at least in the case of the gypsy moth) can be stockpiled for a "one preseason release per site" treatment program.

The success of an egg release program hinges on the competitiveness of F_1 sterile insects. Initially, hatch must occur more or less in synchrony with wild insects, and at a time when young, tender foliage is available. Secondly, F_1 sterile larvae must survive to adulthood and develop in synchrony with wild insects. In this study, larval survival and development were examined in gypsy moths from a P_1 wild strain, the NJS strain and 3 irradiation treatments (F_1 sterile NJS larvae).

Materials and Methods

After exposure to 6, 8 or 10 krads as 8-11 day-old pupae, NJS adult males were mated with normal NJS females. The resulting egg masses were chilled (5-6^{o}C) for ca. 150 days and then were allowed to hatch at 25^{o}C. Other eggs were taken from the rearing colony (NJS) or were field-collected from a moderately high-density population (Upton, MA).

Neonates were provided with red oak foliage that was held in water-filled Aqua-piks which had been shoved through the bottoms of 480ml unwaxed paper cups (10 larvae/cup and 60 cups/strain at 23^{o}C).

If NPV infections occurred in a cup within the first 16 days, the larvae in that cup were reared to pupation at 10/cup and 23^{o} (virus-contaminated cups and Aqua-piks were always replaced immediately). Larvae from NPV-free cups were divided into 2 groups. Sixty larvae per strain were placed individually into cups and were reared (as described above) through the remainder of development. The remaining larvae were transferred to 42x42x57 cm high screen cages and (ca. 100 larvae/cage) were placed in an outdoor insectary. In the cages, foliage was left attached to ca. 0.4m terminals that were held in water-filled flasks. Foliage was changed every other day throughout all rearing. Pupae were removed daily and were weighed at 4 days of age.

Results and Discussion

In 1983, ca. 50% of F_1 sterile larvae became established on foliage, as compared to ca. 90% of NJS and P_1 larvae. This year, irradiation did not cause a decline in first instar survival (78.2 - 84.0% for F_1 sterile strains vs. 78.2% for the NJS strain); however, percent establishment was significantly higher ($p < 0.05$) for the Upton strain (87.8%) than for any of the other strains (78.2 - 84.0%)(Fig. 1). The excessive mortality of 1983 F_1 sterile first instars may have resulted from poor diapause conditions; as neonates, these larvae were noticeably sluggish.

This year, overall survival of F_1 sterile larvae was 63-68% as high as P_1 survival and 76-81% as high as NJS survival (Fig. 1). While not ideal, these differences can be counteracted with an operationally feasible increase in overflooding ratios. Further, most mortality was caused by NPV, which might not be a problem in the relatively low-level population for which this technique is targetted (although the release itself might confound this problem by raising population levels).

As in previous rearing tests, development of F_1 sterile larvae was several days slower than that of untreated larvae (Table 1). In contrast to previous tests, development time among F_1 sterile larvae (except for "virus-cup" females) tended to decrease with increased irradiation. F_1 sterile pupae also were generally smaller than their untreated counterparts - while these size differences often were statistically significant (Table 1), we do not believe that they would be biologically significant within the context of an F_1 sterile egg release program.

Among F_1 sterile insects, pupal weight was not related to IR dose. In fact, results of this test indicate that the only benefit of using 6 krads in a suppression program (rather than the 10 krad dose used for eradication) is the higher percent hatch of F_1 6 krad eggs.

Overall, these rearing tests do not provide any evidence to deny the feasibility of developing a successful, operational F_1 sterile egg release program.

Table 1. Development of gypsy moth larvae from a wild strain (Upton, MA), the NJS strain, and three F_1-sterile treatments, when reared on excised red oak foliage by three different methods.

Strain	Male development 1/ n	Male development 1/ mean days as larvae (± S.E.)	Male development 1/ mean pupal weight (mg ± S.E.)	Female development 1/ n	Female development 1/ mean days as larvae (± S.E.)	Female development 1/ mean pupal weight (mg ± S.E.)
Individually Reared						
Upton	17	40.0 ± 0.7 ab	331 ± 16 a	33	47.1 ± 0.6 a	712 ± 18 c
NJS	28	40.3 ± 1.1 a	345 ± 9 a	22	45.7 ± 0.5 a	708 ± 27 c
NJ-6K IR	26	43.8 ± 0.8 bc	309 ± 15 a	9	51.4 ± 0.9 b	636 ± 32 ab
NJ-8K IR	22	44.5 ± 1.2 c	316 ± 28 a	11	51.3 ± 1.7 b	528 ± 22 a
NJ-10K IR	20	41.3 ± 1.0 a	287 ± 13 a	19	47.8 ± 0.6 a	616 ± 21 b
Virus Cups						
Upton	22	36.7 ± 0.9 a	379 ± 11 a	18	42.6 ± 0.6 a	836 ± 27 c
NJS	31	37.3 ± 0.9 a	353 ± 14 a	20	41.2 ± 0.6 a	691 ± 29 ab
NJ-6K IR	37	40.4 ± 0.6 b	343 ± 10 a	51	43.3 ± 1.3 a	681 ± 21 b
NJ-8K IR	39	37.4 ± 0.6 a	323 ± 11 a	15	44.5 ± 1.0 a	746 ± 49 c
NJ-10K IR	12	39.0 ± 1.0 ab	366 ± 55 a	8	47.4 ± 0.9 a	552 ± 67 a
Insectary Cages						
Upton	144	39.5 ± 0.2 a	392 ± 5 c	107	45.4 ± 0.2 a	809 ± 11 c
NJS	83	39.8 ± 0.3 a	414 ± 7 d	94	45.3 ± 0.2 a	846 ± 14 c
NJ-6K IR	66	43.0 ± 0.4 c	351 ± 8 ab	46	48.4 ± 0.5 b	761 ± 23 b
NJ-8K IR	92	41.3 ± 0.3 b	329 ± 7 a	62	47.7 ± 0.5 b	696 ± 17 a
NJ-10K IR	101	41.7 ± 0.5 b	351 ± 9 b	46	46.2 ± 0.4 a	738 ± 22 ab

1/ Within a column and rearing treatment, means followed by the same letter are not significantly different (p 0.05; Student-Neuman-Keuls test).

Project Number: GM 3.2.7
Project Title: Lure Dispenser Installation Study
Report Period: October 1, 1983 - September 30, 1984
Report Type: Final
Project Leader: E.C. Paszek

The Hercon "Gypsy Moth Lure Tape Plus" is the standard pheromone dispenser used in gypsy moth traps. This small 7/8"x1/8" plastic laminate is stapled above the trap adhesive in the delta trap or onto a plant tie which is suspended inside the milk carton trap. The stapling of these small dispensers is a tedious procedure in the assembly of traps. Last year a study was conducted to determine the usefulness of double stick tape for attaching dispensers to inside trap surfaces. Pheromone dispensers were installed using the following 3 types of double stick tapes and then aged outdoors for 2 and 6 week intervals. Bioassays compared moth catch in traps thus baited with the standard freehanging dispenser.

1/ Scotch 3M Double Stick Tape (1/2" wide)
2/ Scotch 3M 410 Flat Stock Liner Double Coated Tape (3/4" wide)
3/ Sears Self Adhesive Mounting Tape (1/2" wide, 1/16" thick polyurethane foam)

The results of the 1983 study were inconclusive and a new study was designed using 1984 lure dispensers4/. The 2 Scotch brand double stick tapes had one side of the dispenser totally sealed onto the tapes. The Sears self adhesive mounting tape was cut into 1-1/4" x 1/4" lengths and the 7/8" x 1/8" long dispenser was attached lengthwise across the width of the tape allowing for 1/3 of it to overlap on each side of the tape. This overlap allowed for air to circulate completely around 2/3 of the dispenser. Twelve treatments (with aging) were bioassayed in milk carton traps. Each treatment was replicated 10 times in a 12 x 10 grid with the traps spaced 50m apart.

1/ 1/2" wide pressure sensitive transparent tape, sticky on both sides, used as a light duty glue, made by Household and Hardware Products Division/3M, 3M Center, Box 33053, St. Paul, Minnesota 55133.
2/ 3/4" wide, opaque sticky on both sides drafting tape made by Scotch brand tape/3M, St. Paul, Minnesota 55144.
3/ 1/2" wide, 1/16" thick white double coated vinyl foam tape used for holding and hanging light-weight paintings, plaques, charts, etc. Sold by Sears Roebuck and Co., Chicago, Illinois 60684.
4/ 1984 Hercon dispensers Lot No. D0274.

Table 1. 1984 (+) disparlure dispenser installation study.

Treatments[1/]	Mean no. moths caught with dispensers aged: 63 Days	30 Days	0 Days
3M Double Stick	110.2	103.9	83.5
Sears Mounting	83.1	99.0	91.8
3M Double Coated	78.2	84.7	84.7
Standard	81.9	104.7	116.2

1/ Treatments: 1. aged 63 days in greenhouse (6/1 - 8/2/84)
Avg. low temp. 27.2°C, Avg. high temp. 37.8°C
2. aged 30 days in greenhouse (7/3 - 8/2/84)
Avg. low temp. 27.3°C, Avg. high temp. 36.7°C
3. 0-day control, stored in freezer.

Treatments followed by same letter not significantly different.

There was no significant difference between any of the dispensers mounted on the 3 self sticking tapes and the standard free hanging dispensers. The most practical of the 3 tapes, the "Scotch 3M Double Stick Tape 1/2 x 250" (6.9 yd) dispenser type roll, sells for $1.49.

The Scotch 3M 410 Flat Stock Liner Double Coated type 3/4" x 36 yd sells for $5.74/roll. This roll has to be cut with scissors. The Sears Self Adhesive Mounting tape 1/2 x 40" (3.3') sells for $0.99/roll. This roll also has to be cut with scissors.

Project Number: GM 4.2.1
Project Title: Suppression of Gypsy Moth Populations with Release of F-1 Sterile Eggs - Maryland, 1984
Report Period: October 1, 1983 - September 30, 1984
Report Type: Interim
Project Leaders: V. C. Mastro & T. M. O'Dell

This project was initiated to evaluate the effectiveness of using releases of F-1 progeny (eggs) of substerile P-1 males x normal females, for the suppression of natural gypsy moth populations. Sterility of these F-1 eggs, noenate dispersal, survival and development of larvae, and competitiveness of adults are described, respectively, in reports GM 4.2-, GM and GM . From these investigations, a P-1 male irradiation treatment dose of 10 krads was chosen to provide nearly complete sterility in the F-1 generation while still providing adequate hatch of F-1 eggs and survival of F-1 progeny.

Four wooded sites were chosen within the Maryland Gypsy Moth IPM Program area near Lanham, Maryland, to conduct the pilot study. Two of these plots were selected as controls, and two for treatment with F-1 egg masses. Criteria for selection of plots were similarities in forest stand type, 1983-84 egg mass density, and 1983 male trapping records.

Preseason egg mass surveys indicated there was an average of 1.5 egg masses per hectare at site 2. Larval and pupal skins were detected at site 5, however no egg masses were found. Male trapping records from 1984 indicate there was a sparse and fairly uniform population in the area where the plots were located. Direct comparison of trap catches cannot be made because traps were not placed in any of the four sites.

On April 17, 1984, F-1 egg masses (i.e. progeny of males irradiated with 10 krads as 8-11 day-old pupae x untreated laboratory females) were seeded into two of these plots (site 5 - ca. 9.9 ha and site 2 - ca. 7.9 ha). Approximately 8,000 egg masses per hectare were released in both plots. Distribution was done by hand, around pre-established sites established on a grid with a 25 meter spacing between points. F-1 egg masses were scattered around host trees at these sites. Where prefered host trees were not available at a release site, the egg masses were simply scattered around the nearest trees. Hatch of native egg masses in the area of the plots had begun on April 16-17, 1984, and buds of the red oak had just begun to expand on the day of release.

Throughout the larval, pupal, and adult stages, samples were collected from the plots to determine the ratio of sterile F-1's to wild insects. All determinations of insect type were based on fertility of matings of collected (unknown) insects with normal laboratory-reared stock. Analysis of these data is proceding and results will be presented in the next annual report.

Table 1. 1983 male trap records and 1984 post-season egg mass density for pilot study F-1 egg mass release plots.

Site	No. of Males Captured 1983[1/]	Post-season Egg Masses/ha[2/]	Treatment
2	72	1.5 ± 2.2	F-1 egg masses
3	102	0	Control
4	101	2.0 ± 2.6	Control
5	58	0	F-1 egg masses

1/ Average number of males captured based on the three traps closest to the site.
2/ 95% confidence interval.

Project Number: GM 4.2.2
Project Title: Evaluation of Competitiveness in F_1-Sterile Neonates: A Walk in the Square Forest
Report Period: October 1, 1983 - September 30, 1984
Report Type: Interim
Project Leaders: V.C. Mastro, D.R. Lance, C.P. Schwalbe

A successful gypsy moth sterile egg release program requires the deployment of highly competitive F_1-sterile insects. Initially, neonates must be capable of moving off the egg mass, locating suitable food and becoming established on foliage. In a previous test (GM 1.2.1, 10/1/80-9/30/81, p. 81), irradiation of the male parent did not appear to affect the rate at which first instars dropped from oak foliage (i.e. attempted airborne dispersal). This study was undertaken to evaluate the ability of F_1-sterile neonates to move off the egg mass and subsequently locate and climb vertical objects.

Materials and Methods

Four plots (ca. 25m in diameter) were cleared in a wooded area within the National Cemetary on Otis AB. Trees and understory were removed, but the litter was disturbed as little as possible. Six and twelve vertical square posts (9x9cm, 1.2m high) were evenly spaced along 2 concentric circles of 5m and 10m radii, respectively.

Gypsy moth eggs were dehaired, counted and placed into 15cm-diameter petri dishes in the centers of the plots. For several days, hatch and neonate dispersal were allowed to progress naturally, although the petri dishes were covered at night to prevent predation. On sunny days, 30cm diameter plastic sunshades (each supported by three 20cm high rods) were used to prevent overheating of the eggs. The 9x9cm posts were checked every 15 minutes throughout most of the day (normally 0900-1900 h); all larvae on the poles were counted and removed. At the end of the test, unhatched eggs were returned to the laboratory and counted to determine the number of larvae that had hatched. Tests were run using eggs from matings of substerile NJ males (6, 8, and 10 krads as 8-11 day old pupae) x NJS females (held at 5-6^{o} for ca. 150 days) and from wild populations in Massachusetts (Upton) and Pennsylvania (collected in April, 1984).

Results and Discussion

There were no consistent trends either in the percentages of larvae that failed to disperse or in the percentages of dispersing larvae that located and climbed posts (e.g. Table 1). This may have been due, in large part, to inaccurate estimates of the numbers of larvae that dispersed (see below). The percent of recaptures that occurred in the outer ring (which does not depend on the above estimate) was quite consistent among strains in the 6/11-6/15 replicate; in the 6/28-7/1 replicate, a relatively high percentage of wild-type larvae reached the outer ring (Table 1).

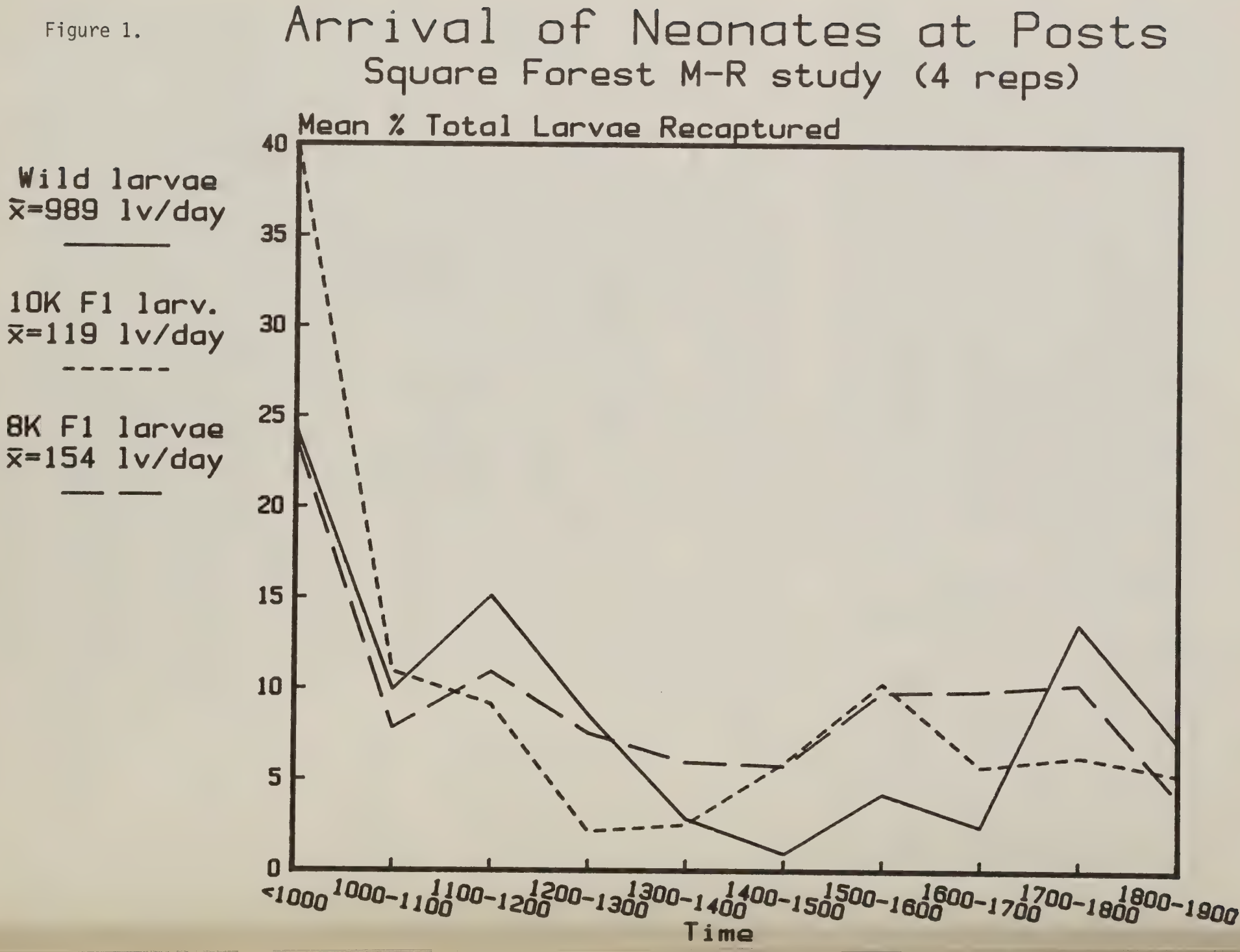
Larvae from all strains were most active in the morning, with a second (smaller) peak of activity late in the afternoon (Fig. 1). This peak appeared to start earlier for F_1-sterile larvae than it did for wild-type neonates, but the available data are not conclusive.

In all, six replicates of this study were set up, but the tests were plagued with two serious (and a number of lesser) problems. First, we had difficulty synchronizing the days of peak hatch among the various strains. As a result, some of the replicates produced meaningful comparisons between only one pair of strains, while other replicates produced no useful comparisons at all. Secondly, the enormous variation in percentages of larvae locating posts (Table 1 and unreported data) suggests that we need an improved method of counting eggs. Initially a volumetric technique was used; this was replaced with the tedious method of counting individual eggs. Either counting technique probably would have provided suitable estimates if most of the eggs hatched during any given trial, but often only 5 - 30% of eggs hatched for any given treatment during the test period. As a result, relatively small percentages of error in the numbers of eggs (which could have been compounded by using both "before" and "after" counts) would have translated into large percentages of error in the numbers of larvae that hatched. Also, large numbers of eggs had to be counted in short periods of time, and accuracy may have been sacrificed for speed in some cases. Lesser problems included an inconsistency of technique and chronic vandalism. Alleviation of these troubles should result in more useful data from the 1985 field season.

Table 1. Dispersal of F_1-sterile and wild neonates in circular plots.

Strain	n of larvae that hatched	% of larvae not dispersing	% dispersing larvae locating posts	% recaptures on outer circle
		11-VI-84 to 15-VI-84		
Wild	7844	4.3	48.3	21.5
10K F_1 sterile	1710	10.4	25.0	21.7
8K F_1 sterile	3099	4.7	25.8	19.9
6K F_1 sterile	3135	5.6	12.8	21.6
		28-VI-84 to 1-VII-84		
Wild	1503	2.1	37.0	41.3
10K F_1 sterile	507	23.9	82.4	33.0
8K F_1 sterile	928	16.1	51.5	22.2

Figure 1.


Arrival of Neonates at Posts
Square Forest M-R study (4 reps)
Mean % Total Larvae Recaptured
Wild larvae
x̄=989 lv/day
10K F1 larv.
x̄=119 lv/day
8K F1 larvae
x̄=154 lv/day
0
5
10
15
20
25
30
35
40
<1000
1000-1100
1100-1200
1200-1300
1300-1400
1400-1500
1500-1600
1600-1700
1700-1800
1800-1900
Time

Project Number: GM 4.2.3
Project Title: Modification of Male Gypsy Moth Behavior by the Pheromones of Other Species
Report Period: October 1, 1983 - September 30, 1984
Report Type: Final
Project Leaders: D. R. Lance, C. P. Schwalbe and V. C. Mastro

When the pheromones of two moth species are deployed in the same trap, capture of one species may be affected by the presence of the other species' pheromone (see PPSD 4.1.1). For example, capture of male gypsy moths declined ca. 80% when Adoxophyes orana (ADOX) pheromone dispensers were added to (+) disparlure-baited milk carton traps. This finding was somewhat unexpected, since the ADOX pheromone (a 9:1 blend of Z-9:Z-11 tetradecenyl acetates) is structurally and taxonomically unrelated to disparlure (cis-7, 8-epoxy-2-methyl octadecane). A series of field studies was undertaken to get a better understanding of this phenomenon.

Study #1. Relative effects of the two isomers.

Disparlure-baited milk carton traps were placed out in a grid of 5 lines of 12 traps each. On each line, 3 of the 12 traps contained various amounts (see Table 1) of the ADOX 9:1 blend on a cotton dental roll. Six of the remaining 9 traps were baited with 3 concentrations each of the 2 isomers. The remaining 3 traps contained only disparlure; one of these was used as a control treatment and the other 2 were placed at the ends of the lines in an attempt to alleviate any "edge effect". Tests were run in a naturally occurring gypsy moth population. On each of the 3 consecutive days of the test, trap capture was recorded, cotton dental rolls were replaced, and (except for end traps) trap positions were rerandomized.

The results of this test are summarized in Table 1. Tested separately, both the Z-9 and Z-11 isomers reduced trap catch; however, at comparable concentrations, the Z-11 appears to be the more potent inhibitor.

Table 1. Effect of Adoxophyes orana pheromone components [1/] on capture of male gypsy moths in (+) disparlure-baited milk carton traps.

Concentration of components (ug/trap) Z-9	Z-11	n of observations	Mean no. of gypsy moths captured [2/] (males/trap/day)
0	0	15	85.3 ± 11.7 a
9	0	15	54.5 ± 10.6 abc
90	0	15	32.5 ± 6.6 d
900	0	15	15.6 ± 2.4 e
0	1	15	80.7 ± 5.9 ab
0	10	15	42.3 ± 5.6 cd
0	100	15	13.3 ± 1.7 e
9	1	15	53.3 ± 7.9 bc
90	10	15	32.3 ± 6.0 d
900	100	15	11.9 ± 1.8 e

1/ Z-9 and Z-11 tetradecenyl acetate; A. orana pheromone is a 9:1 blend, respectively, of these 2 isomers.

2/ (+ S.E.). Means not followed by the same letter are significantly different ($p<0.05$) as determined by the Student-Neuman-Keuls test. Data were subjected to ln(n+1) transformation prior to ANOVA; actual means are shown.

Study #2. Orientation of male gypsy moths to traps containing ADOX pheromone.

Observers were stationed ca. 5 m from traps that contained the standard gypsy moth lure as well as 1 mg of ADOX pheromone, 0.9 mg of Z-9, 0.1 mg of Z-11, or an empty cotton dental roll. The observers recorded the number of males coming within 1 m of the traps, the number orienting to the traps, and the number touching the traps. In one series of observations, the observers also recorded the number of males that actually entered the traps (these tests were run in a naturally occurring gypsy moth population). In a second series of observations, the entry ports were screened shut and observers recorded the length of time that males spent "walking while wing-fanning" prior to leaving the trap (these tests were run with released NJS laboratory-reared males).

Traps were arranged in a circle with a 20 m intertrap spacing (70 m diameter for tests with wild males; laboratory-reared males were released from a center point 15 m from traps). The tests were run blind, and positions of observers were rotated every 10 minutes. Trap positions also were rotated on a regular basis.

The results of these observations (Table 2) suggest that at least a large proportion of the inhibitory effect of ADOX pheromone occurs at some distance from the trap rather than right at the entry port; indeed, the effect was detectable at a distance of 1 m.

Table 2. Orientation of male gypsy moths to (+) disparlure-baited milk carton traps that also contained *Adoxophyes orana* pheromone components [1].

Source of males [2]	Concentration of components (ug/trap) Z-9	Z-11	n [3]	Mean no. of males approaching within 1 m [4]	Mean no. of males orienting to trap [5]	Mean no. of males touching trap [5]	Mean no. of males entering trap [5]	Mean time wing-fanning on trap (sec.) [4]
wild	0	0	24	6.4 a	2.1(33% a)	1.1(54% a)	0.8(70% a)	-
wild	0	100	23	4.9 b	1.3(27% a)	0.5(40% a)	0.3(66% a)	-
wild	900	0	22	4.3 b	1.2(28% a)	0.4(33% a)	0.3(77% a)	-
wild	900	100	23	4.7 b	1.1(23% a)	0.3(28% a)	0.1(43% a)	-
NJS/LR	0	0	16	12.0 z	9.4(78% y)	6.9(74% y)	-	17.5 z
NJS/LR	900	100	16	7.8 z	3.0(38% z)	0.9(30% z)	-	18.3 z

1/ Z-9 and Z-11 tetradecenyl acetate; *A. orana* pheromone is a 9:1 blend (respectively) of these isomers.

2/ Wild = males in a naturally occurring gypsy moth population; NJS/LR males were reared in the laboratory and released in the center of the plot. See text for details.

3/ Number of 10 minute observation periods.

4/ Means that are not followed by the same letter are significantly different ($p<0.05$) as determined by the Student-Neuman-Keuls test. Data for NJS/LR males were analyzed separately.

5/ Within each column, percentages are based on data in the preceding column; those that are not followed by the same letter are significantly different ($p<0.05$; contingency table analysis). Data for NJS/LR males were analyzed separately.

Study #3. Potential use of Adoxophyes orana pheromone as a disruptant for gypsy moth mating.

The disruptant properties of ADOX pheromone were tested in a series of 30 disruption mini-plots (see Figure 1). Potential disruptants were applied to cotton dental roll wicks that were hung from strings (1.5 - 2.5 m high) in naturally occurring gypsy moth populations. Five milk carton traps were placed on stakes (1 m high) in each of the plots. These traps were checked daily for 6 days. Disruptant wicks were replaced every second day.

In 3 control plots, we captured a mean of 45 males/trap/day (Table 3). As a disruptant, racemic disparlure yielded a significant, dose-dependent reduction in capture. In contrast, ADOX pheromone showed no disruptant properties either by itself or in combination with racemic disparlure.

Table 3. Capture of male gypsy moths in disruption mini-plots.

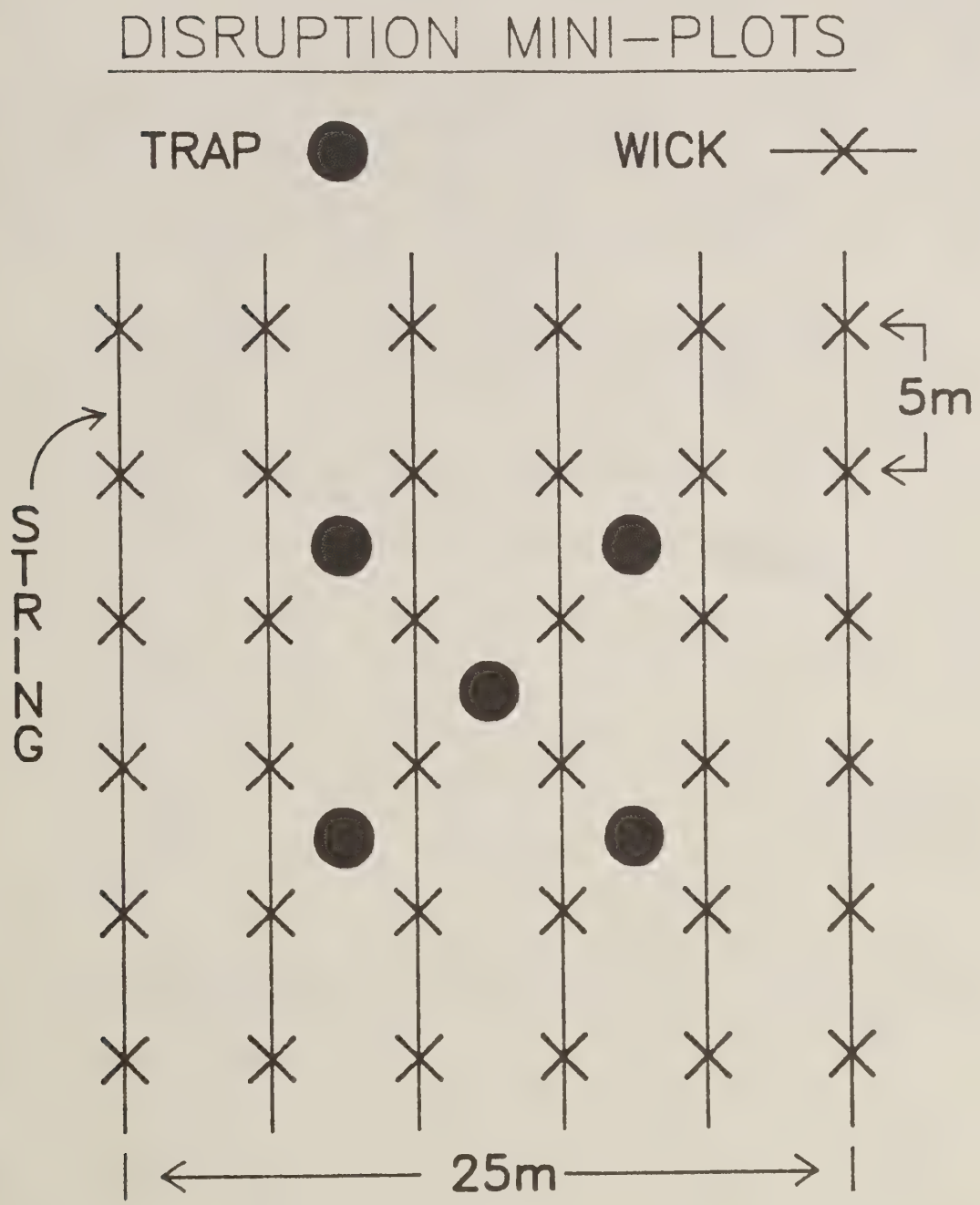
Concentration of potential disruptant (g/ha) 1/		Mean males per trap per day 2/	Adjusted males per trap per day 2,3/
GM	ADOX		
0	0	40.0 ab	43.7 ab
0.04	0	49.3 a	39.5 ab
1.27	0	17.2 abc	21.9 bc
40.0	0	8.5 bc	7.6 d
0	0.04	47.2 abc	39.9 ab
0	1.27	62.2 a	51.9 ab
0	40.0	49.3 a	60.4 a
1.27	0.04	16.7 abc	18.7 bc
1.27	1.27	10.6 abc	12.4 cd
1.27	40.0	4.5 c	16.1 cd

1/ GM = racemic disparlure; ADOX = 9:1 blend of Z-9:Z-11 tetradecenyl acetate.

2/ Means that are not followed by the same letter are significantly different ($p<0.05$), as determined by the Student-Newman-Keuls test. Data subjected to ln(n+1) transformation prior to ANOVA; actual means are shown.

3/ Adjustment based on trap capture on the day prior to the deployment of disruptant compounds.

Figure 1. Layout of plots used for determining disruptant properties of insect pheromones. Strings were hung at 1.5 - 2.5m; wicks (3.5cm cotton dental rolls) containing potential disruptants were clipped to the strings.



Project Number: GM 4.2.4
Project Title: Bioassay of Aged and Unaged Samples of 1981-1984 Hercon Disparlure Dispensers
Report Type: Final
Report Period: October 1, 1983 - September 30, 1984
Project Leader: Edward C. Paszek

Pheromone dispensers are used in traps to survey large areas for gypsy moth infestations. These dispensers are stored in a freezer prior to use and are annually tested in a field bioassay. In the bioassays conducted in 1981-83 it was observed that the new, unaged dispensers (directly out of the freezer) always captured fewer moths than similar dispensers aged for a few weeks outdoors. Accordingly, dispensers probably should be aged for a minimum of a week before they are used, to maximize attractiveness.

In this study, samples of the 1981-1984 disparlure dispensers were individually pierced onto common pins and hung on a tack board, on a shaded east wall inside the greenhouse. They were aged for 7 days (7/10-17/84) at temperatures that averaged a low of 26.6°C and a high of 38.1°C. The unaged controls were stored in a freezer. These dispensers were placed in milk carton traps and bioassayed in a 8x6 grid 50m apart in North Falmouth.

Table 1. Bioassay of aged on unaged 1981-1984 disparlure dispensers on inventory.1/

Year - Lot No.	Mean No. of Males Caught	
	Aged	Unaged
1984-D0274	28.7 ab	27.9 ab
1983-D0053	41.7 a	10.3 cd
1982-D0032	32.6 ab	7.7 de
1981-D0351	15.4 bc	4.1 e

1/ Treatments followed by same letter not significantly different at 5% level. Analysis performed on log males (log 10 transformation of males); however actual means reported here.

The 1981-1983 aged dispensers were significantly different from the unaged dispensers. They outcaptured the unaged dispensers 4 to 1. Surprisingly, there was no significant difference between the aged and unaged 1984 dispensers. The 1984 dispensers were used in 2 other studies where they were aged in the greenhouse for periods of 30 and 63 days. Again, in those studies there were no significant differences in the number of moths captured between the aged or unaged dispensers. Why the 1984 dispensers did not require aging to become active is unexplainable. Possibly the plastic laminate used in making the 1984 dispensers is not identical to the laminates used for the dispensers made 1981-1983.

Inventory of (+) disparlure dispensers in freezer 2/27/85:

Year	Color Code	Number
1984	Aqua	350
1983	Beige	750
1982	Aqua	450
1981	Beige	1450

Project Number: GM 4.2.5
Project Title: Bioassay of Gypsy Moth Pheromone Formulations
Report Period: October 1, 1983 - September 30, 1984
Report Type: Final
Project Leader: E.C. Paszek

In 1983, aged and unaged Albany[1/] hollow fiber disparlure dispensers, (plus GM-1, plus GM-45, plus GM-80) and Sinclair-Rush, septa dispensers were compared to the standard aged and unaged Hercon[2/] laminate dispensers. All of the Albany formulations captured significantly fewer moths than the aged Hercon dispensers. It was recommended not to use these dispensers for the general program in 1984.

A new series of Albany (+) disparlure formulations were bioassayed in 1984. Hollow fiber and PVC septa dispensers were aged for 30 days in the greenhouse[3/] and compared to similarly aged 1984 Hercon laminate dispensers. The Albany standard cotton wick (100ug) and the USDA standard Schwartz-Plimmer cotton wick (100ug) were also used as controls. These formulations were bioassayed 7/23-26/84 in milk carton traps randomly placed 50m apart in a 14 x 8 grid. Each treatment was replicated 8 times.

Table 1. Comparison study of Conrel fibers, PVC septa and 1984 Hercon laminated (+) disparlure dispensers.

Attractant	Mean No. of Males Captured/Trap	
	Unaged	Aged 30 days
Albany Standard (100mg)	36.03 a	
USDA Standard (100mg)	35.38 a	
1984 Hercon	36.38 a	34.53 a
Albany GM 20 End	17.13 b	11.59 bc
Albany GM 10F Center	6.66 c	11.16 bc
Albany GM 10F End	9.81 bc	10.59 bc
PVC-1	11.47 bc	9.58 bc
PVC-2	15.53 bc	8.78 bc

1/ Albany International sold their Pheromone Division in 1984 to Pest Select, Phoenix, Arizona.
2/ 1982 Hercon (Lot D0032) aged outdoors 30 days prior to bioassay.
3/ Greenhouse temperatures 6/23 - 7/23/84, avg. low 26.5°C, avg. high 36.7°C.

Treatments followed by same letter not significantly different at 5% level. Duncan's procedure performed on L Males [UG 10(Males + 1)], however, actual mean value (males) reported here.

The Albany standard, 100ug on cotton wick, USDA standard, 100ug on cotton wick and the aged and unaged 1984 Hercon laminated were not significantly different. However when Albany's hollow fiber and PVC Septa dispensers were used as carriers of the (+) disparlure, they captured significantly fewer moths than the similarly aged and unaged 1984 Hercon laminate dispensers. Since none of Albany's dispensers bioassayed are aqual to Hercon's laminates, they can not be recommended for use in the 1985 survey program.

Project Number: GM 7.3.6
Project Title: Insect Production and Distribution
Report Period: September 1, 1983 - October 30, 1984
Report Type: Interim
Project Leaders: J.J. Baker and John Allen Tanner

The primary objectives of the rearing facility are to produce sufficient quantities of all gypsy moth life stages for the support of projects at this laboratory and at research institutions in the United States and abroad, and to produce sufficient quantities of gypsy moth eggs for future projects.

This year was the first attempt at producing large numbers of F_1 egg masses for field release in connection with the sterile male program. New methods were adopted along the way and the rearing period was July 23, 1984 to January 10, 1985. In all, 650,000 egg masses were produced and are presently in storage.

Table 1. The number of eggs, cups and liters of B-4 diet used to provide gypsy moth life stages for in-house and cooperative program.

	Eggs Infested	B-4 Diet Liters	Number Cups
ARS	1,752	4,994	58,770
Cooperative Program	85,440	441	4,860
Colony	260,620	2,806	33,377
Insecticide	952,596	1,059	18,510
Irradation Study and Sterile Male QC Testing	987,360	5,245	61,710
Sterile Male Behavior and Mass Trapping Evaluation	194,961	1,008	11,869
F_1 Sterile Male - Plain Diet	2,808,000	14,917	175,500
F_1 Sterile Male - Red Diet	403,200	2,142	25,200
Strontium Chloride Testing	295,680	1,570	18,480
Miscellaneous	460,768	6,591	20,590
Total	6,450,377	40,773	428,866

Table 2. Distribution of reared insects, 1984.

Project	Egg Masses	L-2	L-3 L-4	L-5 L-6	Male prepupae	Male pupae	Female prepupae	Female pupae
W. McLane (APHIS)	300	410,880						
V. Mastro (APHIS)					44,677	211,549	24,605	546,312
R. Carde (U. Massachusetts)	185					3,800		2,000
P. Barbosa (U. Maryland)	405		5,330		8,080			
J. Kennedy (England)	68					610		610
M. Ma (U. Maryland)			600	3,500				
C. Yin (U. Massachusetts)	160		5,100					
R. Chianse (New Jersey Dept. of Agriculture)	4,725							
R. Fusco (Pennsylvania FPM)	690							
W. Yendol (Pennsylvania State Univ.)	450							
J. Appleby (Illinois)	160	-	-	-	-	-	-	-
S. Haynes (Maryland)	390	-	-	-	-	-	-	-
TOTAL	7,533	410,880	11,030	3,500	52,757	215,959	24,605	548,931

Table 3. Summary of sterile male production, FY 1984.

	Male prepupae	Male pupae	Female prepupae	Female pupae
Mass reared F_1 sterile male plain	7,581	660,526	3,142	555,878
Mass reared F_1 sterile male red	100	84,450	100	69,400
Sterile male behavior and mass trapping evaluation	5,034	21,536	4,278	13,407
Strontium chloride testing	200	73,650	300	65,100
Total	12,915	840,162	7,820	703,785

Table 4. The number of eggs produced in FY 1984.

FY	Number of eggs
1984	59,857,980

Project Number: GM 1.3.1
Project Title: Evaluating the Development and Reproduction of Insects Produced in the Otis Methods Development Center Rearing Facility
Report Period: October 1, 1983 - September 30, 1984
Report Type: Interim
Project Leaders: John Allen Tanner, J.J. Baker, and Marjorie Palmeri.

Introduction:

The purpose of this project is to monitor the development of the laboratory strains and to determine if it falls within the acceptable range. Data are collected for each strain/generation and are used to detect changes from normal development so that corrective action can be taken.

Budgetary restraints have forced us to limit collection of developmental data to those insects used to maintain each strain (colony). Currently only the New Jersey strain is being maintained but in the near future we hope to add several wild or near wild strains.

Developmental data are collected only on egg eclosion, larval development, female pupal weights, sex ratio, pupae and spin yields, adult female emergence and deformity, and female fecundity. The data are collected using the methods described by Tanner et.al. (1983). The formula used to determine the sex ratio has been changed to the following:

$$\text{Sex Ratio} = \frac{\text{\# male pupae + spins}}{\text{\# female pupae + spins}}$$

The yield of colony eggs/colony mating was also calculated. This gives a reasonable estimate of the fecundity of the colony. It reflects not only the ability of the adults to emerge and mate but also the number of eggs the females can deposit.

With the exception of larval "straggling" there were no major surprises in the development of the New Jersey Colony. This years report was broken down by generation and each generation tended to give similar development data. The 26th generation had a slightly lower survival rate from the egg to pupal stage as well as a lower percentage of females depositing egg masses than the other generations (Table 1 and 2). However, females from this generation tended to deposit more eggs. Also the number of colony eggs per colony mating was higher in the 26th generation (Table 2).

An interesting observation is the oscillation of the mean larval stage on the 10th P.E.I.D. between a higher and a lower value with each succeeding generation (Table 1). The mean larval stage was 2.03 in the 24th generation but dropped to 1.90 in the 25th. It rose to 2.06 in the 26th generation only to drop to 1.83 thus far in the 27th generation.

Larval "straggling" or "stunting" is the one major concern in our rearing program. The condition is characterized by the lack of or reduction in growth in some newly hatched larvae. An individual rearing cup may contain early, mid and late stage larvae as well as pupae. At times the reduction in pupal yields may exceed 40% (personal observation). Those larvae in the early or mid stage never reach the pupal stage unless they are transferred to fresh diet. Electron microscopic examination of the stunted larvae has shown that these larvae contain a Rickettsia-like organism (Bell, personal conversation). At present we have not determined how wide-spread this organism is in our colony or if it is actually causing the straggling. However, anecdotal observations indicate a causal relationship.

Table 1. Performance data of New Jersey colony insects.

Parameter	Generation 1/ 2/ 24 (n=18)		25 (n=36)		26 (n=34)		27 (n=13)	
	$\bar{x}$	sd	$\bar{x}$	sd	$\bar{x}$	sd	$\bar{x}$	sd
% Embryonation	96.2	2.4a	95.1	4.7a	95.5	3.2a	95.2	1.8a
% Hatch	88.1	5.3a	84.3	7.8a	83.7	7.0a	83.8	5.8a
Mean larval stage at 10th P.E.I.D. 3/	2.03	0.13a	1.90	0.20b	2.06	0.21a	1.83	0.27b
Female pupal weights (gm)	2.43	0.19a	2.46	0.20a	2.49	0.24a	2.43	0.14a
% Survival of eggs to pupae	64.9	7.1a	59.2	7.9ab	54.1	14.5b	58.1	10.3ab
Sex Ratio M:F	1.3:1	0.2a	1.2:1	0.1a	1.3:1	0.3a	1.2:1	0.1a
% Adult Female								
Emergence	93.5	6.4a	96.4	4.6a	94.4	4.8a	93.7	5.2a
Deformity	8.6	4.9c	12.6	7.6d	3.7	7.0b	0.5	1.2a

1/ Generation 24 started November 2, 1982 thru February 28, 1983.
Generation 25 started March 7, 1983 thru November 7, 1983.
Generation 26 started November 14, 1983 thru July 2, 1984.
Generation 27 started July 9, 1984 thru October 1, 1984.

2/ Means within a row not followed by the same letters are significantly different at the .05 level (Duncan's Multiple Range Test).

3/ P.E.I.D. = post egg infest day.

Table 2. Reproductive data of New Jersey colony insects.

	Generation 1/ 2/							
	24 (n=18)		25 (n=36)		26 (n=34)		27 (n=13)	
	$\bar{x}$	sd	$\bar{x}$	sd	$\bar{x}$	sd	$\bar{x}$	sd
% of Females Depositing Egg Masses	88.4	10.2a	88.2	7.5a	81.5	9.8b	90.1	6.8a
# Eggs Deposited per Female	1052	115b	1099	118b	1203	250a	1093	79b
# Colony Eggs per Colony Mating	855	122b	946	98a	964	130a	922	42ab

1/ Generation 24 started November 2, 1982 thru February 28, 1983.
Generation 25 started March 7, 1983 thru November 7, 1983.
Generation 26 started November 14, 1983 thru July 2, 1984.
Generation 27 started July 9, 1984 thru October 1, 1984.

2/ Means within a row not followed by the same letters are significantly different at the .05 level (Duncan's Multiple Range Test)

References Cited

Tanner, J.A., Bernadine P. Weeks and Marjorie Palmeri. 1983. Evaluating the Development and Reproduction of Insects Produced in the Otis Methods Development Center Rearing Facility. APHIS Laboratory Report, October 1, 1982 to September 30, 1983: 127 - 135.

Project Number: GM 3.3.1
Project Title: Development and Evaluation of Improved Rearing Techniques
Report Period: October 1, 1983 - September 30, 1984
Report Type: Interim
Project Leaders: John Allen Tanner, J.G.R. Tardif, J.J. Baker, Valerie Douville, and Robert Demanche.

This project is concerned with the development and evaluation of new rearing techniques and the improvement of presently used techniques. Unreliable or inefficient techniques will be modified or discarded.

The F_1 sterile male program is now the major user of reared insects. Currently, we can provide up to approximately 35,000 pupae/day, however, these numbers will have to be increased considerably in the future. To do this, the rearing facility will have to be enlarged and those procedures requiring manual labor will have to be automated.

Currently, the packaging of newly harvested pupae for holding until irradiation and/or mating is done at each harvesting table. It is envisioned that time and space could be saved by transporting the pupae by conveyor belt to a central packaging area. One person would count and package the pupae while the remaining personnel would concentrate on harvesting.

A conveyor system has been constructed to move the pupae to a common collecting area. At the time of this report, the whole system had not been completed; however, the movement of the pupae by the conveyor belt system was tested. The results showed that movement of the pupae by the conveyor belt had no adverse effect on adult emergence rates, nor did it effect the percentage of deformed adults (Table 1). More information will be presented in later reports.

Table 1. Adult emergence and deformity in pupal harvesting systems employing conveyor or manual pupal movement.

Sex	Method of Movement	Percent Emergence	Percent Adult Deformity	Percent Partially Emerged
Male	Conveyor	99.5	0.7	0.2
	Hand	99.3	3.2	0.2
Female	Conveyer	96.4	6.8	1.1
	Hand	96.7	6.9	2.1

A second area open for mechanization is dehairing of the F_1 masses prior to release in the field. Approximately 650,000 egg masses were produced during the 1984 program. All these masses must be dehaired near the date of release. The volume alone makes it impractical to use the hand method described by Tardif and Secrest (1970).

A prototype mechanical dehairing apparatus was developed and tested during the 1983-84 rearing year. This apparatus consisted of a rotating wire drum housed in an evacuated bin. The wire drum had baffles on the inside to help mix the eggs. Air is drawn through a side slit into the bin and across the rotating drum by a Cyclone dust collector with after bagger.

Table 2 shows that the mechanical dehairer had no effect on egg hatch. It took the mechanical dehairer 8 minutes longer than the hand method to dehair 50 masses. Adding BB's to the rotating drum dropped the dehairing time to 2 minutes, but it also significantly reduced the egg hatch. Table 3 shows that the eggs can be tumbled up to 323 minutes without any loss in hatch.

A larger prototype mechanical dehairing apparatus was tested and found to efficiently remove the hairs from approximately 7400 egg masses in one hour. A thorough description of the dehairing apparatus will be presented in the next annual report after the apparatus has been used in the 1985 F_1 sterile male program and a final design agreed upon.

Table 2. The percent hatch of eggs dehaired in the prototype mechanical dehairer with and without BB's.

		Mechanical Dehairer[1/]	
	Control[2/]	Without BBs	With BBs
Percent Hatch[3/]	94.5 ± 3.1a	97.2 ± 1.7a	70.4 ± 9.2b
Time[4/] (min)	5.0	13.0	2.0

1/ ± S.E.
2/ Hand Dehaired
3/ Means within a row not followed by the same letter are significantly different at the .05 level.
4/ Time to dehair eggs.

Table 3. The percent hatch of gypsy moth eggs as affected by the length of time the eggs were rotated in the mechanical dehairer.

	Time (minutes)[1/]					
	23	83	143	203	263	323
Percent Hatch[2/]	96.9±2.7	97.9±1.8	98.0±2.0	97.7±1.8	96.6±2.9	95.7±2.9

1/ ± S.E.
2/ Means were nonsignificant at the .05 level.

Dehairing 7400 egg masses/hour will still require up to 3 weeks to dehair the 650,000 masses on hand. This means that some dehaired eggs will have to be held for 3 or more weeks in a refrigerator. The following test was conducted to determine how long we can hold dehaired eggs under refrigeration.

The F_1 sterile male eggs are held in two different refrigerators. One refrigerator (automated) is programmed to run between 7-8°C, the other refrigerator (walk-in) runs between 3.4-4.5°C. Fertile egg masses are also held in these refrigerators and were used in this test. Eighty egg masses were selected from each refrigerator after 120, 150, or 170 days of chilling. The masses from the same refrigerator were collectively dehaired. Ten samples of eggs (50 eggs/sample) were taken from each group of dehaired eggs and incubated at 23-25°C and 80-90% relative humidity. Each group of remaining dehaired eggs was divided into two subgroups. One subgroup was returned to the automated refrigerator the other to the walk-in refrigerator. This resulted in the following four treatments:

Treatment No.	Location of Chilling	
	Masses	Dehaired Eggs
1	Automated	Automated
2	Automated	Walk-in
3	Walk-in	Walk-in
4	Walk-in	Automated

Ten samples of eggs (50 eggs/sample) were taken weekly from each treatment for 6 weeks. The eggs were incubated as described above. Newly emerged neonates were counted and removed from the hatch dishes daily.

Statistical analysis has not been completed, however, several trends can be identified. The percent hatch of egg masses chilled at 3.4-4.5°C dropped with each increase in the chilling period from 120 to 170 days (Fig.1). Those masses chilled at 7-8°C show only a slight drop in the percent hatch.

Egg masses chilled 120 to 170 days at 3.4-4.5°C showed a marked reduction in hatch as they were held longer at 3.4-4.5°C after dehairing (Fig. 2, 3, 4); holding at 7-8°C did not prevent the reduction in hatch. Egg masses chilled 120 to 170 days at 7-8°C, then dehaired and held an additional 42 days at 7-8°C showed only a slight reduction in hatch. Unfortunately those eggs could not be held long at 7-8°C before a considerable amount of hatch began in the refrigerator. Table 4 summarizes the visual estimates of hatch that occurred during chilling. Dehaired eggs from masses chilled 150 days at 7-8°C could only be held up to 28 days before the hatch was so heavy that samples could not be drawn. Dehaired eggs from masses chilled 170 days could only be held up to 14 days. The premature hatch can be prevented by placing the dehaired eggs at 3.4-4.5°C, however, the dehaired eggs from masses chilled 170 days showed a marked reduction in hatch with increased exposure time to 3.4-4.5°C (Fig. 4).

The hatch of F_1 sterile eggs normally ranges between 30-40%. Any loss in hatch, either through mortality or premature hatch will reduce the effectiveness of this program. Until the optimum temperatures for chilling egg masses and the holding of the dehaired eggs can be determined, it is recommended that the egg masses be chilled at 7-8°C and that the dehaired eggs be held at 3.4-4.5°C. It is also recommended that egg masses chilled 170 or more days should not be dehaired, if possible, until just prior to release.

Table 4. A visual estimate of the amount of hatch occurring within the refrigerator when the egg masses and dehaired eggs were held at 7-8°C

Days egg masses chilled	Days dehaired eggs chilled						
	0	7	14	21	28	35	42
120	None	None	Low	Low	Low	Moderate	Moderate
150	Low	Low	Low	Moderate	Complete	Complete	Complete
170	Low	Heavy	Complete	Complete	Complete	Complete	Complete

References Cited

Tardif, J.G.R., and J.P. Secrest. 1970. Devices for Cleaning and Counting Eggs of of the Gypsy Moth. Journ. Eco. Ent., 63:678-679.

Figure 1. The percent hatch of non-dehaired New Jersey egg masses as affected by the length of their exposure to either 3.4-4.5°C or 7-8°C.

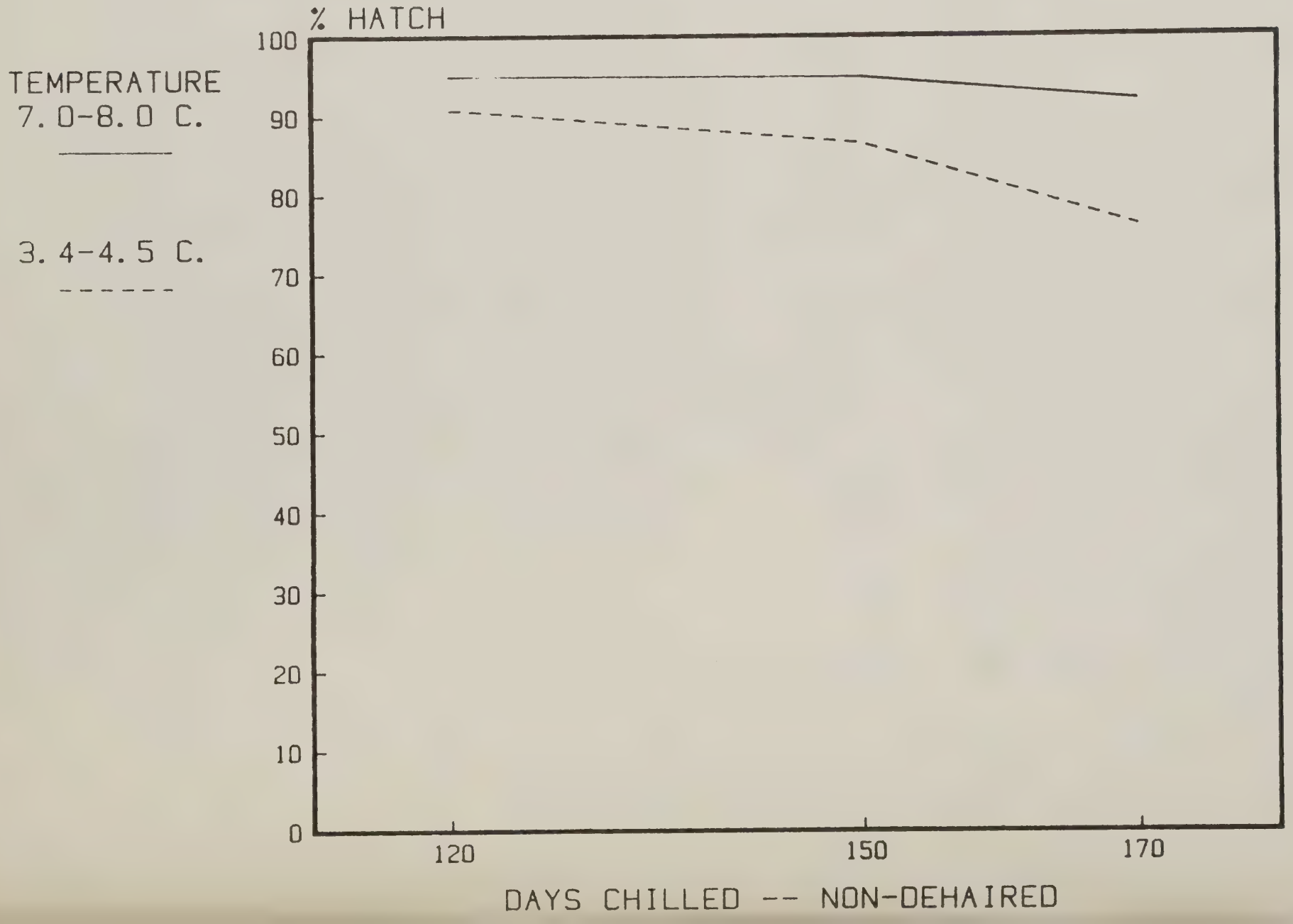


Figure 2. The percent hatch of dehaired New Jersey strain gypsy moth eggs held up to 42 days at either 3.4-4.5°C or 7-8°C after an initial chilling of 120 days as egg masses at 3.4-4.5°C or 7-8°C.

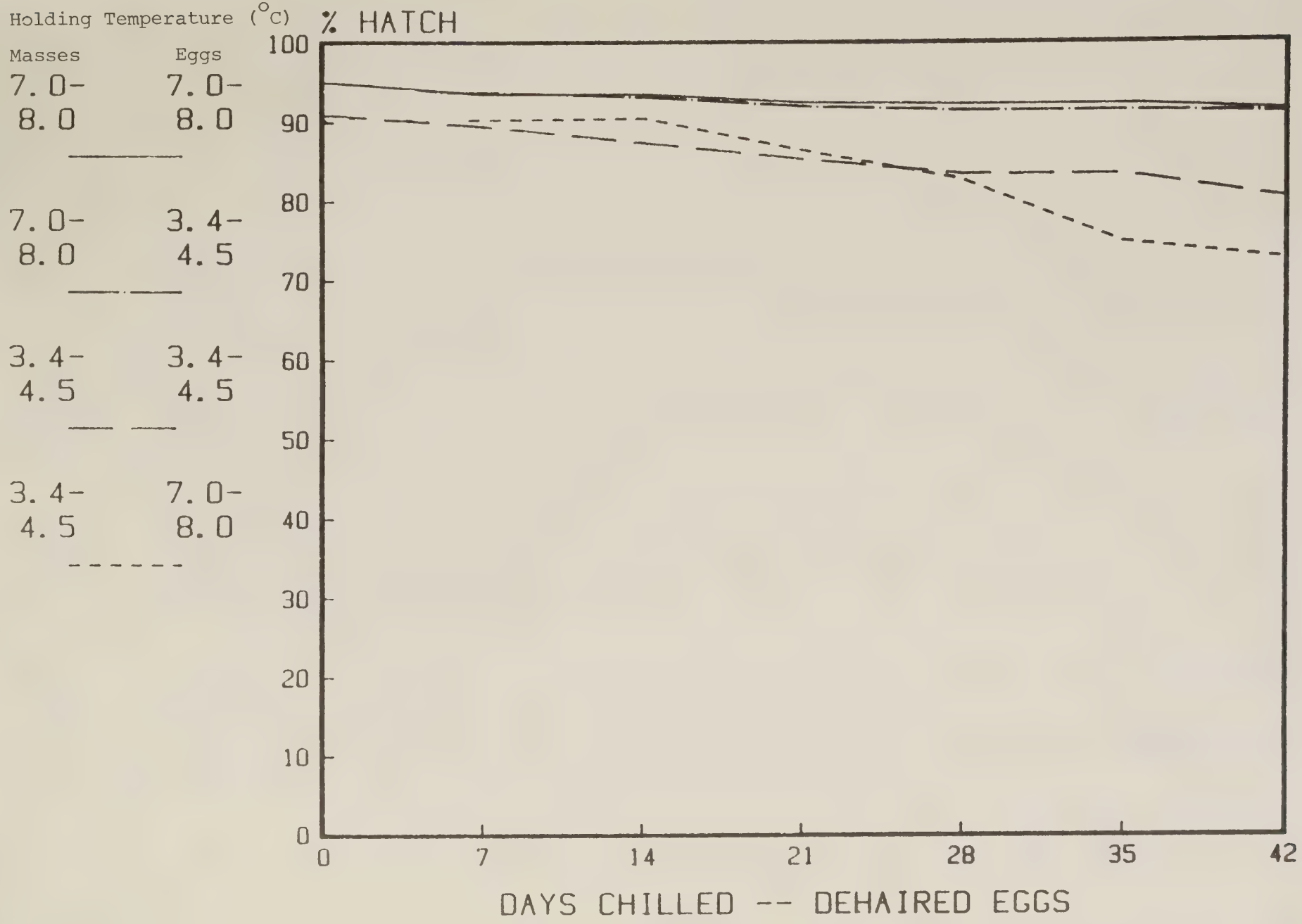


Figure 3. The percent hatch of dehaired New Jersey strain gypsy moth eggs held up to 42 days at either 3.4-4.5°C or 7-8°C after an initial chilling of 150 days as egg masses at 3.4-4.5°C or 7-8°C.

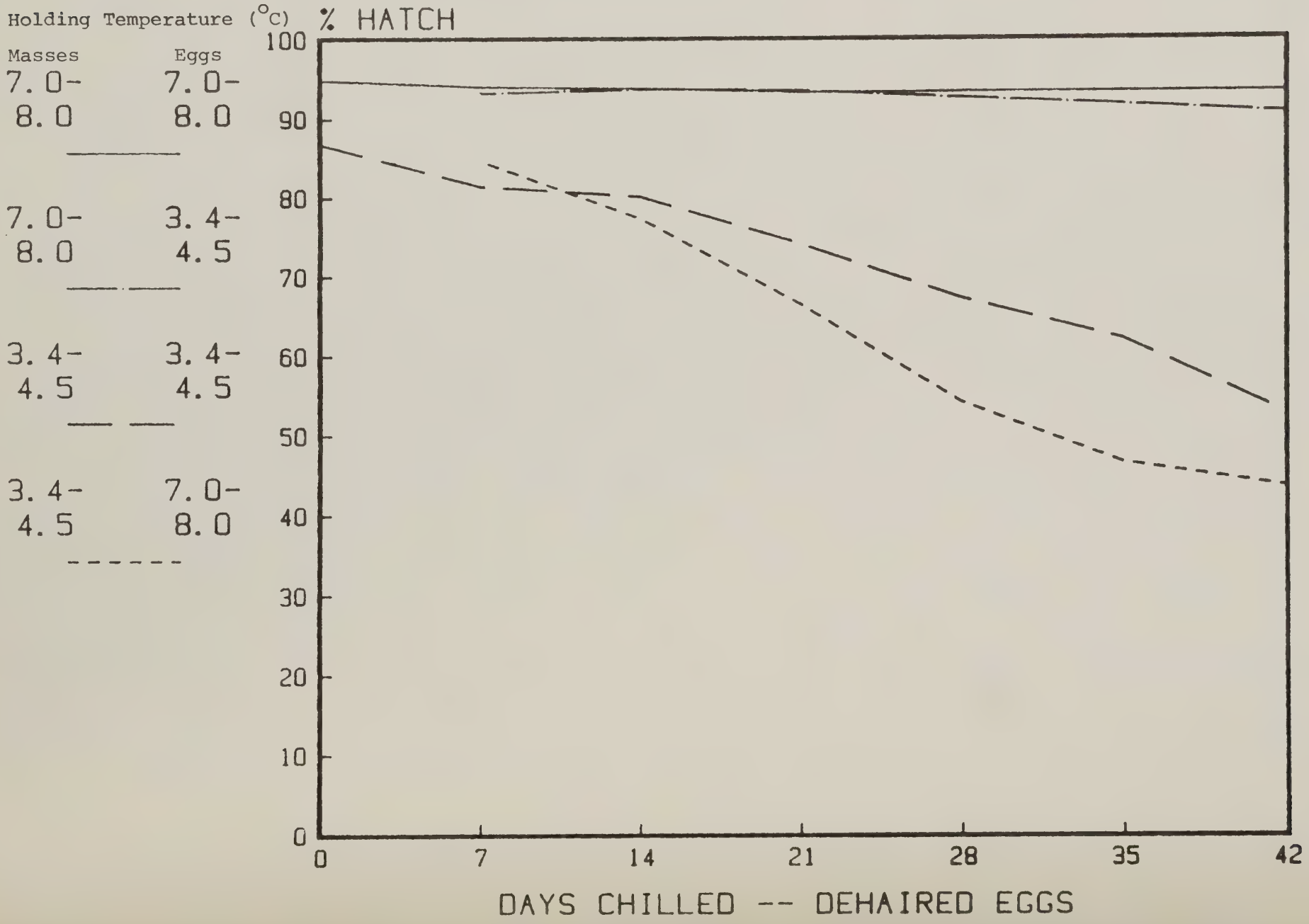
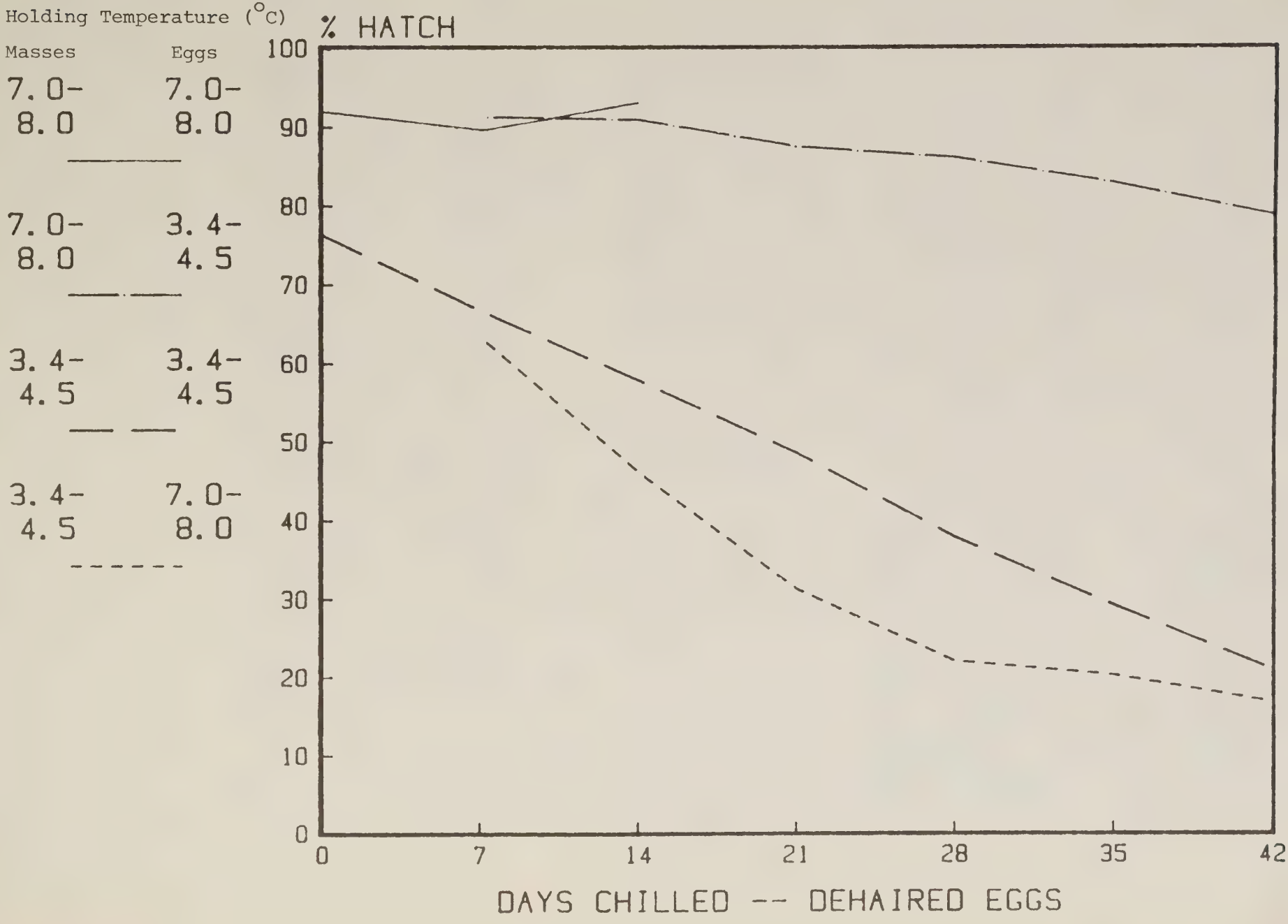


Figure 4. The percent hatch of dehaired New Jersey strain gypsy moth eggs held up to 42 days at either 3.4-4.5°C or 7-8°C after an initial chilling of 170 days as egg masses at 3.4-4.5°C or 7-8°C.



Project Number: GM 4.3.1
Project Title: The Number of Instars in the New Jersey Standard Strain
Report Period: October 1, 1983 - September 30, 1984
Report Type: Interim
Project Leaders: John Allen Tanner, Robert Demanche and Valerie Douville

Introduction

In the F_1 sterile male program, male and female pupae are routinely harvested four days after 10% male pupation has occurred (Tanner and Weeks, 1981). Early in the 1984 rearing program, it was noticed that more female larvae (therefore, fewer pupae) were present at the time of pupal harvest than had been observed in the 1983 program. This resulted in a considerable reduction in the number of F_1 matings (since few females were available) and quickly forced the abandonment of the "10% male pupation plus four days" harvest trigger. In order to meet the production schedule a time consuming "double harvest" technique was implemented (each container was harvested twice, at 7 day intervals).

The New Jersey laboratory strain used in the F_1 sterile male program was observed to produce few six-instar type female larvae (Bell, personal communcation). However, in recent feeding tests using oak foliage, this strain produced a considerably greater number of six instar type female larvae (Mastro, personal communication). The following test was conducted to determine if the New Jersey strain female larvae may be reverting back to the six instar type larvae normally found in nature and to determine if this extra instar may be delaying the onset of pupation. This test was also set up to determine if the extra instar could be induced by exposing the larvae to fresh diet during their development.

Materials and Methods

Surface disinfected and dehaired New Jersey F_{27} eggs were placed into 30 6-oz. fluted cups containing 85 ml of B-4 diet. Newly hatched neonates were transferred into individual 1-1/2 oz. Thunderbird cups containing 24 ml of B-4 diet. Each cup was checked daily and after each molt, the head capsule and cast skin were removed.

In the first replication, one half of the larvae were placed onto fresh diet (24 ml) after the fifth molt to determine if offering the larvae fresh food would induce further molts. In the second replication the changing of the diet was more complex. The diet was changed after each molt from the 1st to 5th molt, 2nd to 5th molt, 3rd to 5th molt and after the 4th and 5th molt. In other treatments the diet was changed once after the 1st, 2nd, 3rd, 4th or 5th molt.

Results

Table 1 shows the percentage of 6 and 7 instar type female larvae as affected by the different feeding techniques. Normally the diet is not changed during the larval period, and insects thus reared had fewer 6 and 7 instar type female larvae in the first replication but not in the second replication. Overall, placing the larvae on fresh diet during its larval developmental period does not seem to increase the percentage of 6 and 7 instar type female larvae. The percentage of 6 and 7 instar type female larvae does seem to vary considerably between replications. Each replication was started with a different batch of eggs and this may indicate that the number of 6 and 7 instar type female larvae may vary considerably from day to day.

The overall differences observed between the male and female pupation time was not due to different developmental rates. Considerably more female larvae went through a 6th and (occasionally) 7th instar. Five instar type female larvae took only 0.9 days longer to pupate than 5 instar type male larvae (Table 2). However, whereas over 92% of the male larvae in these tests were of the 4th and 5th instar type, only 67% of the female larva were (Table 3). Six instar type female larvae took 8.3 days longer to pupate than 5 instar type male larvae while a 7 instar type female larvae took 24.1 days. Clearly, extra instars result in dramatically longer developmental time.

Table 1. The percentage of 6 and 7 instar type female larvae as affected by exposure to fresh diet after molting.

Treatments Diet changed after:	Replications Rep. 1	 Rep. 2
Molts 1 thru 5		43
Molts 2 thru 5		17
Molts 3 thru 5		50
Molts 4 thru 5		40
1st molt		46
2nd molt		31
3rd molt		50
4th molt		41
5th molt	33	40
Diet not changed	16	36

Table 2. The number of days to pupation of 4, 5, 6, and 7 instar type male and female larvae

Sex	Number of Instars 1/ 4	5	6	7
Males	27.4 ± 1.1 2/	31.0 ± 3.0	44.1 ± 10.1	54.0 ± 7.4
Females	32.0 ± 0.0 3/	31.9 ± 2.0	39.3 ± 4.2	55.1 ± 8.4

1/ All means within a column were nonsignificant at the .05 level

2/ ± S.E.

3/ Only one female larvae was of the fourth instar type.

Table 3. The percentage of male and female larvae pupating after the 4th, 5th, 6th and 7th instar.

Sex	Number of instars prior to pupation 4	5	6	7
Male	3.7	88.0	5.8	2.4
Female	0.3	67.0	28.1	4.6

Conclusion

Male and female larvae with the same number of instars tended to pupate at the same time. Feeding larvae fresh diet at various times during their development did not increase the number of instars. The delay in the overall female pupation experienced during the 1984 F_1 sterile male program was probably due to the presence of a higher percentage of 6 and 7 instar type female larvae in the population than in the 1983 program.

Further sampling of the New Jersey colony is necessary to determine the variation in the percentage 6 and 7 instar type female larvae. Methods must also be developed to syncronize male and female development, perhaps by reducing the number of 6 and 7 instar type female larvae. Females that pupate after 5 instars will be smaller and less fecund, but this may be an acceptable trade-off to eliminate the expensive double harvesting necessary to maximize recovery of properly aged male and female pupae.

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Tanner, J. A., and B. P. Weeks. 1981. Development of Mechanical Egg Infestation Procedure. APHIS Laboratory Report, October 1, 1980 to September 30, 1981: 248-256.

Project Number: GM 4.3.2
Project Title: Development of Strontium as a Marker for Gypsy Moth Larvae
Report Period: October 1, 1983 - September 30, 1984
Report Type: Interim
Project Leaders: John Allen Tanner, Larry L. Herbaugh, Don Burns, Valerie Douville and Robert Demanche

Introduction

The gypsy moth sterile male program involves the release of F-1 eggs which develop into sterile adults. These eggs are produced by mating normal females to males exposed to substerilizing dosages of radiation in the pupal stage (Lance et al, 1983). A critical need for this program is a method of marking the F-1 insects so they can be monitored after release.

Metals such as strontium, rubidium and cesium have been successfully used as markers in adult Lepidoptera and Diptera by incorporating the metal directly into the larval diet (Van Steenwyk et al 1978, Moss and Van Steenwyk 1982, 1984, Burns et al 1983, Legg and Chiang 1984). The purpose of this project is to determine if strontium is maternally transmitted to F-1 eggs and how long it can be detected in F-1 larvae.

Methods and Materials

The non-diapausing "Hoy" strain was used to compress the time for obtaining results. Newly eclosed larvae were placed into individual 1-1/2 oz. cups containing B-4 diet with various concentrations of $SRCl_2$. The concentrations of $SRCl_2$ used in the first replication were 0 (Control), 0.05, 0.1, 0.5 and 1.0 percent. In the second replication the 0.5 and 1.0 percent concentrations were replaced by 0.075 and 0.25 percent.

The larvae were reared at 25 $\pm$ 2.0°C, 50-55% RH, and a 16L:8D photoperiod. Adults from the same concentrations were mated. The F-1 egg masses were held at 25 $\pm$ 2.0°C, a 16L:8D photoperiod and high humidity. Core samples of eggs were taken from each mass and used in hatch tests. The remaining eggs were watched daily until the commencement of hatch. All hatch was removed daily. Neonates that hatched on the 1st, 2nd, 4th, 5th, 7th, 8th, 10th, 11th and 12th day were frozen for later analysis. Some of the neonates (50/mass) that hatched on the 3rd, 6th and 9th days were placed onto $SRCl_2$-free diet in 6 oz. cups to determine how long the $SRCl_2$ is retained in the tissue after feeding and growth has commenced. This was accomplished by freezing samples of larvae at periodic intervals until pupation.

All the frozen samples were processed at the USDA-APHIS Methods Development Laboratory in Phoenix, Arizona, using an inductively coupled plasma-atomic emission spectrometry (ICP-AES). The method used was similar to that described by Burns et al 1983.

The effects of placing New Jersey colony eggs directly onto $SRCl_2$ diet, as measured by the percent hatch and the percent establishment of the neonates was also examined. New Jersey eggs were treated in a 10% formalin solution prior to dehairing. The dehaired eggs were then placed mechanically into 6 oz. cups containing 0 (control), 0.05, 0.075, 0.1, or 0.25 percent $SRCl_2$ diet. Ten days later, the percent hatch and percent establishment were determined for each cup.

Results

Only the data from the first replication have been analyzed at this time. $SRCl_2$ concentrations above 0.1% were detrimental to the neonates (Table 1). Survival to the adult stage at 0.1% $SRCl_2$ was similar to the control but at 0.5% and 1.0%, none of the neonates survived to the adult stage. The number of days from the initial placement of neonates onto diet till adult male emergence was also similar to the control for the two lower concentrations. (Table 2). The time to female adult emergence was slightly longer on 0.1% $SRCl_2$ diet than on control diet or diet containing 0.05% $SRCl_2$. The weight of male pupae from 0.1% $SRCl_2$ diet was higher than those from the control and 0.05% $SRCl_2$ diet, however, the weight of female pupae from 0.1% $SRCl_2$ diet was lower than those from the control and 0.05% $SRCl_2$ diet (Table 3). The percentage of females with pupal deformity was considerably lower for larvae reared on $SRCl_2$ diet than control diet (Table 4), but the percent adult deformity was the same for all three diets (Table 5). Egg mass weights (Table 6) and the percent hatch of the eggs (Table 7) were similar between the control and $SRCl_2$ diets.

Placing New Jersey eggs directly onto $SRCl_2$ diet had no effect on the percent hatch of the eggs, or the percent establishment of the neonates (Table 8).

Figure 1 shows the parts per billion (ppb) of $SRCl_2$ found in neonates from the first three days of hatch. Initially, neonates from parents fed higher concentrations of $SRCl_2$ had higher amounts of $SRCl_2$ in their tissues. As the neonates fed on $SRCl_2$-free diet the concentrations of $SRCl_2$ decreased rapidly before leveling off at about the fourth feeding day.

Based on this information, about 50,000 pairs of adults were reared on diet containing 0.1% $SRCl_2$. The resulting egg masses are now being chilled and will be used in 1985 sterile male field tests.

Table 1. The percent survival of gypsy moth larvae to the adult stage as affected by the concentration of $SRCl_2$ in the diet.

	Concentration of $SRCl_2$ (Percent) 1/				
	0 (Control)	.05	.1	.5	1.0
Percent survival	90 a	91 a	91 a	0 b	0 b

1/ Means within a row not followed by the same letter were significantly different at the .05 level.

Table 2. The effects of $SRCl_2$ on the number of days required by gypsy moths to develop from neonates to adults.

	Concentration of $SRCl_2$ (Percent) 1/2/		
	0 (Control)	.05	.1
Males	49.6 ± .3 a	50.1 ± .5 a	49.2 ± .4 a
Females	49.9 ± .3 a	49.9 ± .4 a	51.7 ± .6 b

1/ ± S.E.
2/ Means within a row not followed by the same letter were significantly different at the .05 level.

Table 3. Gypsy moth pupal weights (gm) as affected by the concentration of $SRCl_2$ in the diet.

	Concentration of $SRCl_2$ (Percent) 1/2/		
	0 (Control)	.05	.01
Males	0.503 ± .010 a	0.529 ± .019 a	0.554 ± .012 b
Females	1.840 ± .060 a	1.857 ± .055 a	1.749 ± .064 b

1/ ± S.E.
2/ Means within a row not followed by the same letter were significantly different at the .05 level.

Table 4. Gypsy moth pupal deformity as affected by the concentration of $SRCl_2$ in the diet.

	Concentration of $SRCl_2$ (Percent)		
	0 (Control)	.05	.01
Males	3.8 a	5.3	0.0
Females	79.7 a	58.6	60.8

1/ Means within a row not followed by the same letter were significantly different at the .05 level.

Table 5. Gypsy moth adult deformity as affected by the concentration of $SRCl_2$ in the diet.

	Concentration of $SRCl_2$ (Percent) 1/		
	0 (Control)	.05	.01
Male	3.8	0.0	0.0
Female	14.1	4.3	9.8

1/ Means within a row were not significantly different at the .05 level.

Table 6. Gypsy moth egg mass weights (mg) as affected by the concentration of $SRCl_2$ in the diet.

	Concentrations of $SRCl_2$ (Percent) 1/2/		
	0 (Control)	.05	.01
Egg mass wt. (mg)	494.6 ± 37.2	564.1 ± 25.2	510.0 ± 29.6

1/ ± S.E.
2/ No two means were significantly different at the .05 level.

Table 7. Percent hatch of gypsy moth eggs as affected by the concentration of $SRCl_2$ in the diet.

	Concentration of $SRCl_2$ (Percent) 1/2/		
	0 (Control)	.05	.01
Percent hatch	86.4 ± 24.7	92.9 ± 10.9	95.0 ± 5.9

1/ ± S.E.
2/ No two means were significantly different at the .05 level.

Table 8. The percent hatch of New Jersey Colony eggs and the subsequent establishment of the neonates as affected by placing the eggs directly onto diet containing various concentrations of $SRCl_2$.

	Concentration of $SRCL_1$ (percent) 1/2/				
	0 (Control)	0.05	0.1	0.5	1.0
Percent Hatch	93.5 ± 1.4	92.0 ± 1.2	91.6 ± 1.8	92.7 ± 1.1	93.4 ± 0.8
Establishment	99.1 ± 0.4	99.1 ± 0.5	99.3 ± 0.3	99.5 ± 0.8	98.3 ± 0.8

1/ ± S.E.
2/ Means within a row were not significantly different at the .05 level.

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Burns, D.W., M.P. Murphy, L.L. Jones, M.L. Parsons, P. Farnsworth, E.T. Ozaki, and R.T. Staten. 1983. Evaluation of Internal Elemental Markers for Mediterranean Fruit Fly (Diptera:Tephritidae) Reared on Tagged Artificial Diets. J. Econ. Entomol. 76:1397-1400.

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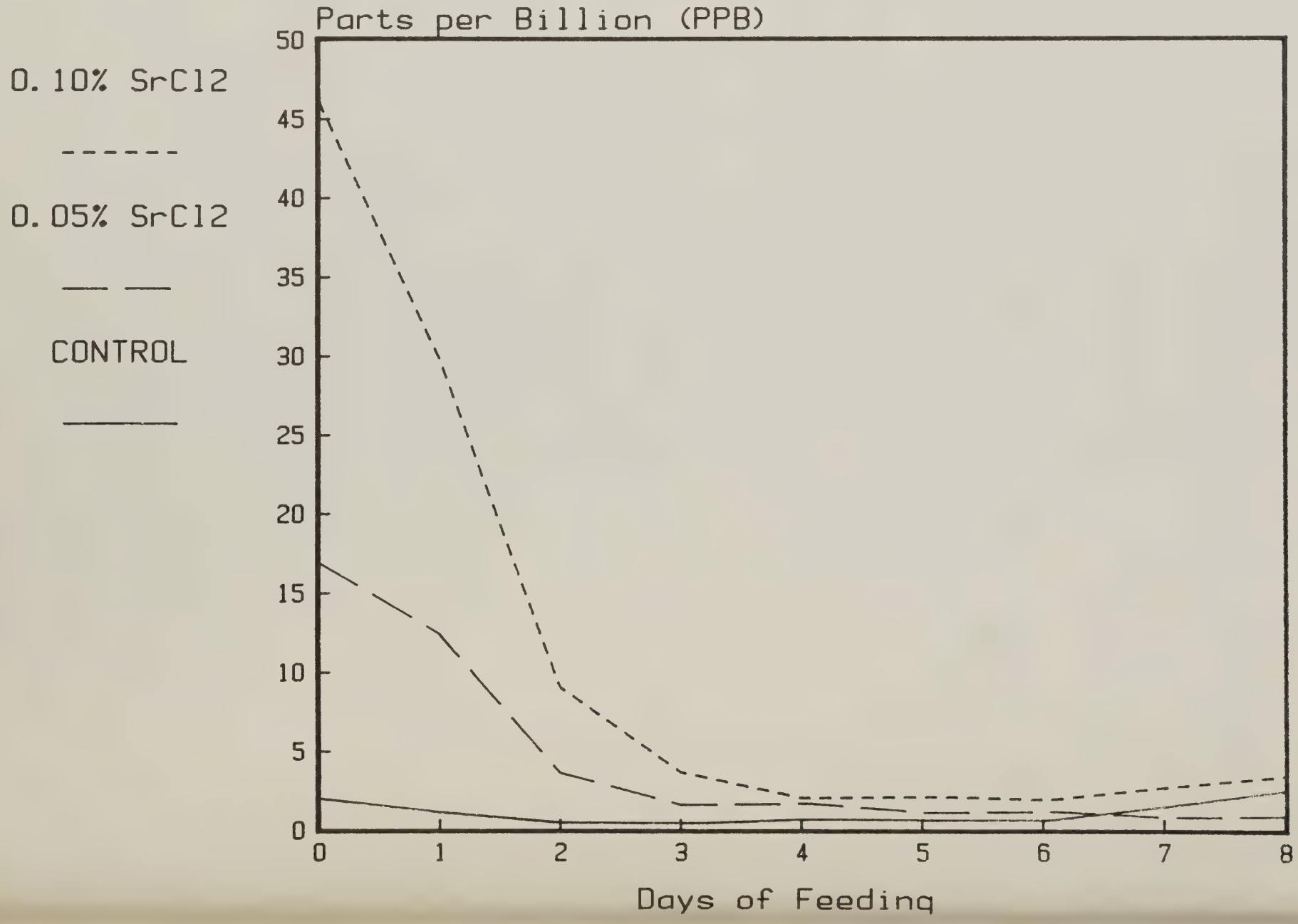
Legg, D.E. and H.C. Chiang. 1984. Rubidium Marking Technique for the European Corn Borer (Lepidoptera:Pyralidae) in Corn. Environ. Entomol. 13:579-583.

Moss, J.I. and R.A. Van Streenwyk. 1982. Marking Pink Bollworm (Lepidoptera: Gelechiidae) with Cesium. Environ. Entomol. 11:1264-1268.

Moss, J.I. and R.A. Van Steenwyk. 1984. Marking Cabbage Looper (Lepidoptera: Noctuidae) with Cesium. Environ. Entomol. 13:390-393.

Van Steenwyk, R.A., G.R. Ballmer, A.L. Page and H.T. Reynolds. 1978. Marking Pink Bollworm with Rubidium. Ann. Entomol. Soc. Am. 71:81-84.

Figure 1. The concentration (parts per billion) of $SRCl_2$ in the tissue of F-1 larvae reared on $SRCl_2$ free diet as affected by the concentration of $SRCl_2$ in the parents diet.



Project Number: CNPPSDP 2.1.1.
Project Title: Methods Development Support of Cooperation National Plant Pest Survey and Detection Program
Report Period: October 1, 1983 - September 30, 1984
Report Type: Interim
Project Leader: Arnold S. Foudin

This year has brought about dramatic changes in the Survey Methods Development Program (SMD). The SMD program has moved into a renovated laboratory facility, with an administrative office and computer room. Our work has been redirected away from survey and detection biology towards standardization of survey methods and pest data imput and output.

Investigations into developing an immunological technique to distinguish between the two important pathogens of corn, Cochliobolus carbonus (commonly known as Helminthosporium carbonum) and Cochliobolus heterostrophus (commonly known as Helminthosporium maydis) were continued. The purpose of this work was to allow field inspection personnel to quickly distinguish between the Drechslera anamorphs of these two species that incite strikingly similar leaf spots and blight in corn. The first step in the development process of a "field immunological test" was the production of large quantities of dry, viable, morphologically normal, and clean conidia (free of contaminating bacteria and fungi). One of the major problems we faced was that the standard growth substrates used for the growth and identification of these two fungi produced relatively few condia which poorly fit the standard mycological description. Our laboratory succeeded in producing a simple-to-prepare and inexpensive substrate for conidial production. A manuscript describing the methodology is currently in preparation. The substrate is a modified lima bean agar made from raw dried lima beans using USP grade agar (cost savings).

In March we started the immunological phase of the development process, working in cooperation with Drs. Catherine Vogelweid and John Berg of the Department of Veterinary Microbiology at the University. Initially two rabbits were injected with a living whole conidial suspension of different concentrations in order to determine the maximum antigen dose the rabbits could sustain. The actual production of the first lot of antisera to the conidia of C. carbonus started in late April. Ten rabbits were employed with a maximum conidial concentration of 6 mg/ml in 0.01% Tween solution. Injections were at one week intervals, and collection was on the 6th week. Detailed information on the general health and reaction of the rabbits as compared to control rabbits was collected at time zero and during the six week period. No abnormal reactions were observed, and hyperimmune serum was collected on May 5, 1984 by Dr. Vogelweid.

In late March Gayle Fry (laboratory technician) started working in the laboratory of Dr. Arthur Karr to learn the procedure involved in counter-immunoelectrophoresis (CIE) and other related immunoelectrophoretic techniques. These techniques were to be used with the rabbit antiserum to identify unique antibodies. The isolated antibodies, unique to the surface antigens of the conidia, were to be employed in a monoclonal antibody system to yield pure antibodies active against the specific fungal pathogen of interest. Initially, efforts to demonstrate activity by a simple conidial agglutination test were unsuccessful, and an alternative method was tried. We tried to physically or chemically strip the antigens from the intact conidia

by simple shaking in water and detergent solution (SDS), but with negative results. More drastic chemical treatment in cold and hot trichloroacetic acid (TCA) was tried, and all of these initial experiments failed to reveal antibody activity in the collected serum. Due to a number of changes in the stated goals of SMD (as put forward by the National Survey Director) and the departure of Mrs. Fry, the work was terminated at this point. We hope to pursue this project in the future.

During FY-83 I submitted to the National Survey Program Director's office a number of verbal reports on the necessity of acquiring a more practical and usable coding system for entry of pest and host names (data elements) into the Cooperative National Plant Pest Survey and Detection Program (CNPPSDP) located on a USDA, mainframe computer at Ft. Collins, Colorado. The CNPPSDP Director thought the coding question important enough to form a standing committee on coding. The committee consisted of chairman Dr. Ken Roach of the Ohio Department of Agriculture, Dr. Ray Hite, APHIS area survey coordinator for the north central states, and Barry Bryce of the Hyattsville Survey staff. The committee met in Columbus, Ohio, and I presented the SMD report on the priority need for a host/pest coding system. The report, based on a study of presently available coding systems and designs, stated that there were only two known systems currently in existence which met most of the twenty-three coding criteria stated in the report (Item 1). One system was the Environmental Protection Agency's National Agricultural Pesticide Impact Registration System coding for pest and host situation (e.g., corn field, corn bin, corn feed) developed over a ten year period by a group headed by Dr. Douglas Sutherland of the EPA. The other system was the United States Soil Conservation Service's coding system for plants. This system only addressed coding for plant (host) species. The outcome of the initial research and reports by SMD was that Dr. Sutherland was very happy to cooperate with the Survey Coding Committee, and following the National Program meeting in October of 1984, the user states and the Hyattsville staff agreed that the EPA coding system will be adopted by the National Survey Program probably in the fall or early winter of 1985.

ITEM 1

CODING CRITERIA DEVELOPED BY THE SURVEY METHODS

DEVELOPMENT LABORATORY FOR PEST/HOST NAMES

1. Cost of the coding system realistic.
2. Flexible enough to cover all possible hosts, pests and combinations.
3. Could it handle races, cultivars, lines, biotypes, pathovars (p.v.) and forma species (f. sp.).
4. System could denote exotic and endemic hosts and pests.
5. Could the coding system handle scientific vs. generic or common names.
6. Was the coding system error prone or would it contain an error checking system.
7. Multi-level system, e.g., common and scientific name with a single code.
8. Limit the number of characters in code to approximately "8".
9. The use of both alpha and numerical characters.
10. Coding carries information on type of pest (e.g. insect, fungus) and some taxonomic information.
11. Temporary coding classification for unidentified pests.
12. Assigned or self-generating coding system.
13. Availability of both computer-stored and hard copy coding information.
14. The existence of a coding manual describing system.
15. Ease of entry of historical pest and host coded data.
16. System is in use and proven.
17. Plain language coding system.
18. Coding system does not create computer storage limitations.
19. Does not contain potential limitations on scope of hosts and pests being considered for entry into the national system.
20. Flexibility of coding system to expand to new uses.
21. User friendly - simplicity.
22. Able to cope with synonomy.
23. Coding system files interact with mainframe DBMS.

In September of 1984, SMD was assigned the task of researching and developing three export certification manuals for the seeds of sorghum, soybeans and dry beans and peas. Sorghum was later replaced by alfalfa seed. We received the names of outside experts, supplied by the American Seed Trade Association through the Hyattsville staff, who were to serve on a committee which would produce the individual export certification manuals. Names of other committee members were also to be supplied (FY-85) by the National Plant Board for inclusion on the committee. The strategy that SMD advocated and with which the committee members concur, is that a copy of the American Phytopathological Society disease compendium for the appropriate crop be included in the manual and that the manual body be of limited size and practical nature with a brief description of how a field or seed inspection is to be conducted, the correct time and method of inspection, cross indexes of country and pathogens of export importance, and common disease names and pathogen names. Where possible, the excellent morphological and symptom description available in the disease compendia would be used, instead of trying to produce a description of our own. Color plates of the disease symptoms in the compendia also would be employed. The information to be in the manual will be placed on the SMD micro-computer, and hard-copy will be generated by means of our word processing capabilities.

In August 1984, in response to complaints that the reports available from the CNPPSDP computer were not always accurate and the implied obligation of SMD to assist with the correction of any biological or output standardization problem, SMD started to generate each of the twelve available reports. Therefore, SMD was the first user to access all twelve report formats. We found numerous technical problems with the report structures and supporting computer software. Working in close cooperatrion with ADSS staff on the FCCC hot-line and other ADSS staff members, SMD was able to pin-point the critical problems in the report structures and formats. A detailed report with recommendations on changes in the FCCC report output has been produced and will be forwarded to the National Survey Staff in early 1985.

In view of the deficiencies in the structure and biological content of the FCCC output pest and host reports discussed at the national meeting of the CNPPSDP, SMD attempted to construct additional software to correct the flaws in the output reports, where possible, and to summarize and tabulate the information into a form which could be interpreted by both APHIS and the user states. We were pleasantly surprised with the progress we made in accomplishing our goal. The most complex and sizable data bases (European Corn Borer and Gypsy Moth Trapping Data), which represented in some cases in excess of 50,000 to over 75,000 lines of information off of FCCC, were condensed into a very usable and informative report. We selected the gypsy moth trapping data report as a model system to examine, because it was representative of many of the problems of the output reports from FCCC and it contained information that was considered to be vital to the affected states and to a national APHIS effort to deal with gypsy moth trapping and eradication. As a result, the CNPPSDP will have in its possession in mid-1985, the software and instructions to enable any one of the Area Survey Coordinators, the National Survey Staff, or SMD to produce a real-time, practical, summary report of data obtained from the FCCC facility. Because of the late entry of some data into the FCCC system, the final 1984 gypsy moth trapping summary report could not be completed until after January, 1985, but because the necessary technical developments and research to carry out this task were performed prior to 1985, an example is included in this 1984 SMD report (Item 2).

With the completion of this short-term project (enhancement of the usefulness and quality of data obtainable from FCCC) in early 1985, we will terminate these activities in anticipation of the acquisition of an operational DBMS for use by the CNPPSCP.

In March 1984, Dr. Paul Teng of the University of Minnesota informed SMD that the major rewrite of his survey standardization data base, PESMID, from PASCAL to COBOL was nearly completed by his then post-doctorate Dr. Baker. Because of the move of Dr. Baker from Minnesota to Utah and subsequent difficulties in loading this material onto the FCCC Univac computer, it was not until late 1984 that PESMID was loaded and operational. Since this period, SMD has obtained loading instructions and data specifications for the placement of survey methods information into the data base. Because of the delay in the decisions relating to the location, type of mainframe computer, DBMS, and even the computer language in which PESMID will finally reside, SMD has delayed actual loading of PESMID survey methods data onto the FCCC Univac computer. Instead, we have constructed a similar file structure for our Zenith micro-computer, and we will start loading survey methods data in early 1985. This, we hope, will eliminate the real possiblity that the data will have to be manually entered twice, once into the FCCC system, and then into whatever system we adopt in late 1985.

As a result of the negative response and numerous comments about the difficulty of completing the 1984 Corn PESMID questionnaires, SMD undertook a study of which crops and pests were most often reported into the CNPPSDP and how SMD could most expeditiously obtain this information for entry into PESMID. We ultimately selected 39 pests which were highly reported on the eight most reported hosts (Item 3). The area survey coordinators were notified of this selection with the permission of the National Survey Director and were asked to solicit the states in their areas for any existing published information pertaining to survey or detection of any one of the 39 selected pests. This information will be forwarded to SMD where it will be abstracted and the information entered into the PESMID data file.

ITEM 2

SUMMARY REPORT OF GYPSY MOTH
TRAPPING FOR THE STATE OF VIRGINIA

LEGEND:

**** = Original report data not obtained from pheromone traps.
** = Missing data.
* = Number of positive traps in original report exceeds number of traps.

Method of Calculation of Positive Traps:

(Total no. of reported traps) - (No. of reported traps)
IF "zero" then (Number of reported positive traps) =
then (Number of reported positive traps)

IF "zero" then (Number of reported positive traps) =
(Total number of reported traps)

(Number/Number) = Number of counties in state/Number of Independent Cities (IC)

(IC) = Independent City

Names of traps accepted as pheromone type traps:

1. Milk Carton
2. Wing-Style
3. Gypsy Moth
4. Delta-Style
5. Delta Pher
6. Pheromone

Names of traps not accepted as pheromone type traps:

1. 15W Blackl
2. Trunk Band

Trapping Report (04): Gypsy Moth
Revision 5 - February, 1985

	Total Traps	Positive Traps	Average per Positive Traps
VIRGINIA (95/41)			
1. Accomack	47	47*	8.29
2. Albemarle	366	47	1.87
3. Alleghany	15	0	0
4. Amelia	86	35	1.54
5. Amherst	15	0	0
6. Appomattox	89	3	1.33
7. Arlington	3	3*	125.66
8. Augusta	231	17	1.82
9. Bath	16	0	0
10. Bedford	65	0	0
11. Bland	14	0	0
12. Botetourt	173	3	1
13. Brunswick	139	23	1.78
14. Buchanan	16	0	0
15. Buckingham	230	26	1.3
16. Campbell	62	0	0
17. Caroline	129	129*	5.45
18. Carroll	124	0	0
19. Charles City	56	9	1
20. Charlotte	123	6	1.16
21. Chesterfield	123	81	2
22. Clarke	15	15*	456.4
23. Craig	20	0	0
24. Culpeper	27	27*	54.37
25. Cumberland	75	30	1.63
26. Dickenson	9	0	0
27. Dinwiddie	130	49	1.44
28. Essex	73	73*	3.57
29. Fairfax	37	37*	156.86
30. Fauquier	27	27*	190.55
31. Floyd	425	0	0
32. Fluvanna	77	25	2.44
33. Franklin	22	0	0
34. Frederick	30	30*	83.76
35. Giles	4	0	0
36. Glouchester	67	67	2.37
37. Goochland	66	53	2.11
38. Grayson	212	0	0
39. Greene	38	25	2.4
40. Greensville	73	9	5
41. Halifax	47	1	1
42. Hanover	126	126*	3.7
43. Henrico	64	64*	2.98
44. Henry	16	0	0
45. Highland	78	0	0

		Total Traps	Positive Traps	Average per Positive Traps
46.	Isle of Wight	83	20	1.85
47.	James City	41	18	1.94
48.	King and Queen	82	82*	3.13
49.	King George	108	86	7.38
50.	King William	63	63*	3.11
51.	Lancaster	45	45*	4.22
52.	Lee	6	0	0
53.	Loudoun	40	40*	92.52
54.	Louisa	266	128	3.64
55.	Lunenburg	92	7	1
56.	Madison	33	33*	6.63
57.	Mathews	31	31*	4.25
58.	Mecklenburg	178	12	1.25
59.	Middlesex	42	42	2.14
60.	Montgomery	374	2	1
61.	Nelson	47	5	1.2
62.	New Kent	60	23	1.3
63.	Northhampton	18	18*	3.38
64.	Northumberland	61	61*	4.4
65.	Nottoway	80	36	1.41
66.	Orange	99	99*	16.92
67.	Page	20	20*	21
68.	Patrick	227	3	1.33
69.	Pittsylvania	54	0	0
70.	Powhatan	69	35	1.65
71.	Prince Edward	83	13	1.84
72.	Prince George	73	26	1.38
73.	Prince William	23	23*	105.17
74.	Pulaski	79	0	0
75.	Rappahonnock	10	10*	84.8
76.	Richmond	55	55*	4.83
77.	Roanoke	112	0	0
78.	Rockbridge	195	2	1
79.	Rockingham	72	14	1.64
80.	Russell	10	0	0
81.	Scott	41	0	0
82.	Shenandoah	22	22*	40.9
83.	Smyth	97	0	0
84.	Southhampton	157	50	1.94
85.	Spotsylvania	228	194	8.36
86.	Stafford	52	52*	32.25
87.	Surry	75	17	1.41
88.	Sussex	120	13	3.15
89.	Tazewell	17	0	0
90.	Warren	15	15*	111.46
91.	Washington	300	6	5

(Item 2 Continued)

	Total Traps	Positive Traps	Average per Positive Traps
92. Westmoreland	142	74	4.56
93. Wise	12	0	0
94. Wythe	99	0	0
95. York	36	36	3.3
96. Alexandria (IC)	6	6*	47.83
97. Chesapeake (IC)	74	26	1.88
98. Hampton (IC)	19	8	1.87
99. Newport News (IC)	20	20*	8.3
100. Norfolk (IC)	17	2	1
101. Petersburg (IC)	9	5	4.6
102. Poquoson (IC)	1	1	9
103. Richmond (IC)	18	18*	7.94
104. Roanoke (IC)	13	1	1
105. Suffolk (IC)	96	36	1.36
106. Virginia Beach (IC)	156	63	4.11
107. Williamsburg (IC)	****		

ITEM 3

Cotton

Bollworm/Budworm
Boll Weevil
Plant bugs

Corn

European corn borer
Western corn rootworm
Smut (symptom)
Leaf blight
Black cutworm

Tobacco

Tobacco hornworm
Tobacco budworm
Green peach aphid
Tobacco mosaic virus

Soybeans

Mexican bean beetle
Green clover worm
Bean leaf beetle
Leaf blight
Downy mildew
Powdery mildew

Alfalfa

Alfalfa weevil
Potato leafhopper
Pea aphid
Leaf spot
Black stem
Anthracnose
Verticillium

Ranger

Grasshoppers
Musk thistle weevil
Black grass bug
Range caterpillar

Apple

Spotted tentiform leafminer
Codling moth
Redbanded leafroller
Apple magot
Fusicladium scab
Fire blight

Wheat

Leaf rust
Stem rust
Green bug
Cereal leaf beetle

Project Number: CNPPSDP 4.1.1
Project Title: Pheromone-Based Survey Technology for Early Detection of Exotic Insect Pests.
Report Period: April 3, 1983 - September 30, 1984
Report Type: Interim
Project Leaders: V.C. Mastro, C.P. Schwalbe, P.C. Kingsley and D.R. Lance.
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Introduction:

The objective of this program is to provide pheromone-based survey technology for detection of introductions of exotic pests. The following report is a summary of all project activities since the project proposal was submitted in April of 1983 (revised July, 1983). Consistent with the goals of APHIS, PPQ, pheromone-baited traps are a very sensitive tool for detection survey of exotic pests. When traps for exotic insects are systematically deployed, and this deployment is incorporated into existing trapping programs, the cost will be minimal while the survey should be sensitive enough to enable detection of introductions while populations are still isolated, small and manageable. As initially proposed, testing was (and is continuing to be) conducted to determine which pheromones are compatible when placed in the same trap. Compatible baits are those that, when placed in the same trap, do not negatively effect capture of any of the target species. This report summarizes all of the pheromone bait combination studies, both domestic and foreign, conducted to date. It also reports on trap design studies which were conducted in concert with bait combination tests. Third, and perhaps most importantly, testing was also done to determine which commercial pheromone formulations are superior in terms of maximizing trap catch.

To determine where operational traps could be baited for multiple species, a current use pattern of pheromone baited traps within the United States was needed. In 1984, all fifty states were surveyed for trap-use information. The results of this survey, which are included in this report, have helped to determine which bait combinations could operationally be useful. This information has influenced which combination of baits are tested for target species response. For example, if only a few traps are placed nationally for a domestic pest, then that species is not a good candidate for bait compatibility studies. Alternatively, programs like the gypsy moth survey employ up to 250,000 traps each year in a variety of situations and this, clearly, offers opportunities for integration of exotic trapping interests.

Finally, in 1984, a set of recommendations (Appendix 5) were developed for survey for seven exotic pests domestically. Pilot-scale surveys for the seven species were conducted nationally, following the recommendations of projected host range. Included in this report is a summary of the outcome of this pilot scale survey (Appendix 5a).

Domestic Studies

Pheromone baits for various exotic species were tested for compatibility with three domestic species which are trapped for on a rather broad scale: gypsy moth, Lymantria dispar; pink bollworm, Pectinophora gossypiella; and the codling moth, Laspeyresia pomonella.

Gypsy Moth, Lymantria dispar

Trial 1. During the 1983 field season, several bait combinations were tested for the gypsy moth in a large field trial. In this trial, traps were placed in straight lines (50m inter-trap spacing). Five complete replicates (lines) of the test were established. All traps were rerandomized daily, for a total of seven readings. Standard high-capacity (milk carton) gypsy moth traps were hung from the limbs of trees at ca. 1.5m in height for this test. Table 1 presents the results of this initial series of pheromone bait combination tests.

Results of this first test series demonstrated that baits for five other species (CM, PFM, LB, PBW, and HZ) can be placed in gypsy moth traps without significantly reducing male captures. In 1983, pheromone baits for two additinal species, which were not available earlier, were tested in combination with gypsy moth bait dispensers.

Trial 2. Pheromone baits for Adoxophyes orana (ADOX) were tested in combination with gypsy moth baits using the same test design as Trial 1, except traps were ramdomized and read 8 times.

Trial 3. In Trial 3, pheromone baits for Spodoptera littoralis were tested in combination with gypsy moth baits but, because native male flight was over, laboratory-reared male moths were released for this trial. A circular plot design was used with trap treatments alternately placed on the circumference (20m inter-trap spacing) of a 70m radius circle. Laboratory-reared males were placed in the center of the plot as pupae and allowed to eclose and disperse. Traps were checked and moved one position each day for a total of 5 readings. Combinations of baits for either A. orana or S. littoralis in traps baited for gypsy moth significantly reduced capture (Tables 2 & 3). Combining A. orana pheromone in traps baited for gypsy moth reduced trap catch most dramatically.

Trials 4a & 4b. In 1984, three additional exotic pheromones were tested in combination with gypsy moth. Initially, a plot was established using the same design as Trial 1 (i.e. 5 replicates, reading and randomization daily, 50m inter-trap spacing). Traps were checked a total of four times in test four and, because native male flight was decreasing and very light by the fourth reading, a second test was initiated (Trial 4b). Design of this test was the same as Trial 3 except that five replicates of each treatment were used. Traps were read and randomized daily seven times. Results of tests 4a and 4b are presented on Table 4. All three combinations reduced male gypsy moth trap captures significantly lower than the control treatment (i.e. gypsy moth bait only).

Table 1. Mean numbers of gypsy moth, Lymantria dispar males captured in traps[1/] baited with L. dispar pheromone in paired combination with various other attractants. Test performed at Otis Methods Development Center, Massachusetts 7/20 - 7/27/83[2/].

Attractant Combination[3/] 1	2	$\bar{x}$ number of L. dispar captured per trap [4/]
GM-her-lam	CM alb-fib	24.94 a b
GM-her-lam	PFM alb-rs	24.60 a
GM-her-lam	Control	20.37 a b c
GM-her-lam	LB alb-rs	18.43 a b c
GM-her-lam	PBW alb-fib	16.34 b c
GM-her-lam	HZ alb-fib	14.60 c
GM-her-lam	FCM alb-fib	9.31 d
GM-her-lam	HP alb-fib	5.77 e
GM-her-lam	CM+HV+HZ	3.79 e f
GM-her-lam	HA alb-fib	3.09 f g
GM-her-lam	PBW+HA+HP+PFM+GVM+FCM	2.11 g
GM-her-lam	HV alb-fib	1.54 g

1/ Standard USDA high capacity (milk carton) gypsy moth traps used for all bait treatments.

2/ Five complete blocks were read and re-randomized seven times = thirty-five observations per treatment.

3/ Species codes, manufacturer codes, and dispenser types in Appendix 1, 2, and 3, respectively.

4/ Means followed by the same letter are not different at the 5% level of significance according to Duncan's Multiple Range Test. Analysis performed on transformed data [log(n+1)]; actual mean values are presented.

Table 2. Mean numbers of gypsy moth, Lymantria dispar, males captured in traps[1/] baited with the pheromone dispensers for L. dispar alone and in combination with pheromone dispenser for Adoxophyes orana. Test performed at Otis Methods Development Center, MA - 1983.

Attractant Combination [2/] (Test 8/4 - 8/18/83 5 blocks - 8 readings)		$\bar{x}$ number of L. dispar captured per trap [3/]
GM-her-lam	Control	7.05 a
GM-her-lam	ADOX-zoe-pc	.98 b

1/ Standard USDA high capacity (milk carton) gypsy moth traps used for all treatments.

2/ Species codes, manufacturer codes and dispenser codes in Appendix 1, 2, and 3, respectively.

3/ Analysis of variance using log(n+1) transformation indicates the treatments are significantly different at the 5% level of significance; actual means are presented.

Table 3. Mean numbers of gypsy moth, Lymantria dispar males captured in traps[1/] baited with dispensers of L. dispar alone and in paired combination with pheromone dispensers for Spodoptera littoralis. Test performed at Otis Methods Development Center, MA - 1983.

Attractant Combination [2/] (Test 8/18 - 8/25/83 11 blocks - 5 readings		$\bar{x}$ number of L. dispar captured per trap [3/]
GM-her-lam	Control	12.69 a
GM-her-lam	ECL zoe-rs	4.75 b

1/ Standard USDA high capacity (milk carton) gypsy moth traps used for all treatments.

2/ Species codes, manufacturer codes, and dispenser codes in Appendix 1, 2, and 3, respectively.

3/ Analysis of variance using log(n=1) transformation indicates the treatments are significantly different at the 5% level of significance; actual means are presented.

Table 4. Mean numbers of gypsy moth, Lymantria dispar males captured in traps[1/] baited with pheromone dispensers for L. dispar alone and in paired combination with pheromone dispensers for various other species. Test performed at Otis Methods Development Center, MA - 1984.

Attractant Combination [2/]		$\bar{x}$ number of males captured/trap/day[3/]	
		Trial 4a[4/] (feral males)	Trial 4b[4/] (laboratory-reared males)
1	2		
GM her-lam	Control	7.45 a	7.35 a
GM her-lam	EA alb-rs	4.65 b	3.20 b
GM her-lam	EP lab-rs	4.05 b	1.85 c
Gm her-lam	NA alb-rs	5.85 a b	1.88 c

1/ Standard USDA high capacity (milk carton) gypsy moth traps used for all treatments.

2/ Species codes, manufacturer codes, and dispenser type codes in Appendix 1, 2, and 3, respectively.

3/ Vertically, means followed by the same letter are not different at the 5% level of significance according to Duncan's Multiple Range Test.

4/ Trial 4-5 replicates read and randomized 4 times; Trial 5-5 replicates read and randomized 8 times.

Table 5. Mean numbers of gypsy moth, Lymantria dispar males captured in traps[1/] baited with L. dispar pheromone alone and in paired combination with five other chemical attractants at five concentrations. Test was conducted at Otis Methods Development Center 8/2/83 - 8/10/83[2/].

Attractant Combination [3/] 1	2	$\bar{x}$ number of gypsy moth males captured/day/trap [4/]
GM her-lam	Control	5.63 c d e
GM her-lam	0.001 mg (Z)-9-C_{16} ALD	8.93 a b c d e
GM her-lam	0.01 mg (Z)-9-C_{16} ALD	8.30 a b c d e
GM her-lam	0.1 mg (Z)-9-C_{16} ALD	8.80 a b c d e
GM her-lam	1.0 mg (Z)-9-C_{16} ALD	9.14 a b c d e
GM her-lam	10.0 mg (Z)-9-C_{16} ALD	8.17 a b c d e
GM her-lam	0.001 mg (Z)-11-C_{16} OH	8.50 a b c d e
GM her-lam	0.01 mg (Z)-11-C_{16} OH	12.17 a
GM her-lam	0.1 mg (Z)-11-C_{16} OH	9.00 a b c d e
GM her-lam	1.0 mg (Z)-11-C_{16} OH	7.67 a b c d e
GM her-lam	10.0 mg (Z)-11-C_{16} OH	5.77 d e
GM her-lam	0.001 mg (Z)-11-C_{16} ALD	10.13 a b c
GM her-lam	0.01 mg (Z)-11-C_{16} ALD	9.10 a b c d e
GM her-lam	0.1 mg (Z)-11-C_{16} ALD	7.50 a b c d e
GM her-lam	1.0 mg (Z)-11-C_{16} ALD	10.30 a b c d e
GM her-lam	10.0 mg (Z)-11-C_{16} ALD	1.07 f
GM her-lam	0.001 mg (Z)-9-C_{14} ALD	11.25 a b
GM her-lam	0.01 mg (Z)-9-C_{14} ALD	7.17 a b c d e
GM her-lam	0.1 mg (Z)-9-C_{14} ALD	8.70 a b c d e
GM her-lam	1.0 mg (Z)-9-C_{14} ALD	8.55 a b c d e
GM her-lam	10.0 mg (Z)-9-C_{14} ALD	6.75 b c d e
GM her-lam	0.001 mg (Z)-11-C_{16} OAC	9.57 a b c d e
GM her-lam	0.01 mg (Z)-11-C_{16} OAC	10.27 a b
GM her-lam	0.1 mg (Z)-11-C_{16} OAC	6.93 b c d e
GM her-lam	1.0 mg (Z)-11-C_{16} OAC	5.77 e
GM her-lam	10.0 mg (Z)-11-C_{16} OAC	2.10 f

1/ Standard USDA high capacity (milk carton) gypsy moth traps used for all treatments.

2/ Five complete blocks were read and re-randomized six times = thirty observations per treatment.

3/ Species codes, manufacturer codes and dispenser types in Appendix 1, 2, and 3, respectively. Chemical nomenclature:
(Z)-9-C_{16} ALD = (Z)-9-Hexadecenal (Z)-11-C_{16} OH = (Z)-11-Hexadecen-1-ol
(Z)-11-C_{16} ALD = (Z)-11-Hexadecenal (Z)-9-C_{14} ALD = (Z)-9-Tetradecenal
(Z)-11-C_{16} OAC = (Z)-11-Hexadecen-1-ol acetate.

4/ Means followed by the same letter are not different at the 5% level of significance according to Duncan's Multiple Range Test. Analysis performed on transformed data [log(n+1)]; actual means are presented.

Trial 5. Results of Trial 1 in 1983, demonstrated that baits for several species (CM, PFM, LB, PBW, AND HZ) did not significantly reduce the number of male gypsy moths captured. However, paired combinations with pheromone dispensers for three of the four Heliothis species tested (HV, HZ, HA, and HP) did significantly reduce the gypsy moth male captures. To determine which pheromone component(s) were acting as inhibitors, and at what levels each of the five compounds used in Heliothis attractants were inhibitory, the five compounds were tested at five concentrations in paired combinations with gypsy pheromone dispensers. Serial dilutions were prepared for each compound with n-hexane, so that 100 ul of solution would contain the appropriate amount. Rubber septa dispensers were charged on the day the test was initiated and were replaced three days later. Plot design was the same as Trial 1. Results of this test are presented on Table 5.

Apparently, for gypsy moth males, the strongest inhibitor is (Z)-11-Hexadecen-1-ol acetate which significantly reduced male captures at both the 1 and 10mg loading rates. At the highest loading rate, (Z)-11-Hexadecanal also acted as an inhibitor and reduced trap catch significantly. The other three compounds, at least at the concentrations we tested, did not significantly reduce trap catch and, in fact, some loadings resulted in numbers of male captures which were significantly greater than the control (i.e. traps baited with gypsy moth attractant alone).

Pink Bollworm, Pectinophora gossypiella:

Trial 6. Bait combination studies with the pink bollworm P. gossypiella, were conducted in Phoenix, Arizona, during the 1983 field season. Plot design was similar to Trial 1, except that rows of traps (replicates), were spaced ca. 20m apart, and the inter-trap spacing was ca. 23m. For placement, traps were hung on stakes so that they were level with the crop height. Traps were read and, within a row, re-randomized daily for a total of 3 readings. There was a total of 7 blocks (rows of traps) which resulted in twenty-one observations for each treatment. Although several bait combinations did not reduce numbers of pink bollworm captured, baits for 3 of the Heliothis species tested were strong inhibitors (Table 6).

Trial 7. Because of the strong inhibitory effect of attractants for these Heliothis species, a separate test of the individual pheromone components (used in Heliothis baits) was initiated. Serial dilutions of four compounds were prepared with n-hexane, so that a 100 ul amount would contain .01, 0.1, 1.0, or 10.0 mg of the chemical. Rubber septa were used to dispense all compounds.

Results of this test, presented in Table 7, indicate that at the highest concentrations tested, three of the four coupounds inhibit pink bollworm male trap capture. Only (Z)-9-Hexadecenal (=(Z)9-C_{16} ALD) did not significantly reduce trap catch. The most powerful inhibitor, (Z)-11-Hexadecen-1-ol acetate, significantly reduced trap capture at all concentrations tested, and at the two highest concentrations, nearly prevented capture of pink bollworm males in traps.

Table 6. Mean number of pink bollworm Pectinophora gossypiella males captured in traps[1/] baited with P. gossypiella pheromone alone and in paired combinations with various other attractants. Test was performed in Phoenix, AZ., 8/26 - 9/3/83[2/].

Attractant Combinations [3/] 1	2	$\bar{x}$ number P. gossypiella males captured/trap/reading[4/]
PBW lab-rs	CM alb-fib	56.90 a
PBW lab-rs	GM her-lam	52.14 a b
PBW lab-rs	HZ alb-fib	52.29 a b
PBW lab-rs	Control	48.76 a b
PBW lab-rs	PFM alb-rs	47.52 a b c
PBW lab-rs	LB alb-rs	44.57 a b c
PBW lab rs	ECL zoe-rs	39.14 b c
PBW lab-rs	FCM alb-fib	31.05 c d
PBW lab-rs	HA alb-fib	21.48 d
PBW lab-rs	HV alb-fib	10.29 e
PBW lab-rs	HP alb-fib	2.43 f
PBW lab-rs	ADOX zoe-pc	1.52 f

1/ Standard USDA orange delta traps used for all treatments.

2/ Seven complete replicates were read and randomized three times = twenty-one observations per treatment.

3/ Species codes, manufacturer codes and dispenser codes in Appendix 1, 2, and 3, respectively.

4/ Means followed by the same letter are not different at the 5% level of significance according to Duncan's Multiple Range Test. Analysis performed on transformed data [log(n+1)]; actual means are presented.

Table 7. Mean numbers of pink bollworm, Pectinophora gossypiella males captured in traps[1/] baited with P. gossypiella pheromone alone and in paired combination with four other chemical attractants at four concentrations. Test was conducted in Phoenix, AZ, 9/5/83 - 9/9/83[2/].

1	Attractant Combination[3/] 2	$\bar{x}$ number of pink bollworm males captured/day/trap[4/]
PBW lab-rs	Control	22.85 a
PBW lab-rs	0.01 mg (Z)-11-C_{16} ALD	17.75 a b c
PBW lab-rs	0.1 mg (Z)-11-C_{16} ALD	17.35 a b c
PBW lab-rs	1.0 mg (Z)-11-C_{16} ALD	16.35 b c d
PBW lab-rs	10.0 mg (Z)-11-C_{16} ALD	5.50 e
PBW lab-rs	0.01 mg (Z)-11-C_{16} OAC	15.90 c d
PBW lab-rs	0.1 mg (Z)-11-C_{16} OAC	2.45 e f
PBW lab-rs	1.0 mg (Z)-11-C_{16} OAC	0.45 f
PBW lab-rs	10.0 mg (Z)-11-C_{16} OAC	0.35 f
PBW lab-rs	0.01 mg (Z)-9-C_{16} ALD	16.35 a b c
PBW lab-rs	0.1 mg (Z)-9-C_{16} ALD	21.00 a b
PBW lab-rs	1.0 mg (Z)-9-C_{16} ALD	16.10 a b c
PBW lab-rs	10.0 mg (Z)-9-C_{16} ALD	23.85 a b c
PBW lab-rs	0.01 mg (Z)-9-C_{14} ALD	23.15 a b c
PBW lab-rs	0.1 mg (Z)-9-C_{14} ALD	14.90 a b c
PBW lab-rs	1.0 mg (Z)-9-C_{14} ALD	12.70 c d
PBW lab-rs	10.0 mg (Z)-9-C_{14} ALD	8.10 d

1/ Standard USDA orange delta traps used for all treatments.

2/ Five complete replicates were read and re-randomized four times = twenty observations per treatment.

3/ Species codes, manufacturer codes, and dispenser types in Appendix 1, 2, and 3, respectively. Chemical nomenclature:
(Z)-11-C_{16} ALD = (Z)-11-Hexadecenal (Z)-11-C_{16} OAC = (Z)-11-Hexadecen-1-ol acetate
(Z)-9-C_{16} ALD = (Z)-9-Hexadecenal (Z)-9-C_{14} ALD = (Z)-9-Tetradecenal.

Codling moth: Laspeyresia pomonella

Trial 8. Domestically, large numbers of traps are placed annually for codling moth (see National Pheromone Trap Use Patterns section of this report, Table 23). Because several of the exotic pests included in this program are orchard pests, combination of their lures in codling moth traps would be desirable. To test the compatibility of pheromones for several exotic species with codling moth pheromone in the same trap, a field test was conducted in an abandoned apple orchard in New York during the 1984 field season. Included in this test were two combinations with domestic species (gypsy moth x codling moth and tufted budmoth x codling moth). These combinations were tested to determine if either of these could be surveyed for simultaneously with the codling moth. Unfortunately, baits for several other exotic species of interest were not available when this test was conducted, however, these paired bait combinations will be tested in 1985.

A wing-type trap (Pherocon-1C) was used to test all bait combinations. Three different configurations of the delta trap were also included in this test to determine if they were suitable for trapping codling moths. For placement, all traps were hung from the limbs of orchard trees at ca. 2m in height. The inter-trap spacing and the spacing between lines of traps (replicates) was 20 meters. Five complete replicates (lines containing all treatments) were established. All traps were checked and randomized three times. Because of rain and cool temperatures, the third reading resulted in very low number of males captured, and this observation is not included in the results presented in Table 8. In fact, because of the low numbers of males captured, and the limited observation, statistical analysis produced poor resolution between the treatments.

The control treatment (codling moth pheromone alone) and base bait for paired combinations was the Albany commercial preparation. Traps baited with this formulation captured significantly more males than the Zoecon Corp. (zoe) preparation. The treatment using the wing-type trap also captured significantly more males than any of the configurations of the delta trap which were tested. Apparently, pheromones for two of the exotic species tested (ADOX and FCM) act as strong inhibitors for codling moth males. However, all combinations will be retested during the 1985 field season in a more extensive series of tests.

African Studies

Several pests native to the African continent rank high in importance as potential threats to American agriculture. Work in the Republic of South Africa was planned to test the compatability of various pheromone baits for five species: false codling moth, Cryptophlebia leucotreta; Old world bollworm, Heliothis armigera; Egyptian cotton leafworm, Spodoptera littoralis; the red bollworm, Diparopis castanea, and the spiny bollworm, Earias insulana. Tests with two of the target species, H. armigera and S. littoralis, were carried out on the O.T.K. experimental cooperative farm with the assistance of Dr. J.D. Mohr of the South African Plant Protection Research Institute. Unfortunately, because of several factors, D. castanea and E. insulana were not abundant enough to conduct pheromone studies at this time.

Table 8. Mean numbers of codling moth, Laspeyresia pomonella, males captured in traps baited with L. pomonella pheromone alone and in paired combination with various attractants. Test was conducted at Sodus, New York, June 12-15, 1984[1/].

Attractant 1	Combination[2/] 2	Trap Type[3/]	$\bar{x}$ number of codling moth males captured/trap/day[4/]
CM alb-fib	Control	P-1C	3.90 a
CM alb-fib	GM-her-lam	P-1C	2.20 a
CM alb-fib	LB alb-rs	P-1C	2.70 a b
CM alb-fib	PFM alb-rs	P-1C	1.10 a b c
CM alb-fib	TAB alb-fib	P-1C	0.80 a b c
CM alb-fib	- - -	br-delta-ec	0.40 b c
CM alb-fib	- - -	or-delta-ec	0.40 c
CM zoe-rs	- - -	P-1C	0.20 c
CM alb-fib	- - -	or-delta-eo	0.10 c
CM alb-fib	ADOX zoe-pv	P-1C	0.10 c
CM alb-fib	FCM alb-fib	P-1C	0.10 c

1/ Five complete replicates were read and re-randomized twice for ten observations per treatment.

2/ Species codes, manufacturer codes, and dispenser types in Appendix 1, 2, and 3, respectively.

3/ Trap type codes, in Appendix 4.

4/ Means followed by the same letter are not different at the 5% level of significance according to Duncan's Multiple Range Test. Analysis performed on transformed data [log(n+1)]; actual means are presented here.

Old World Bollworm - Heliothis armigera:

Trial 9. Preliminary tests indicated that the delta trap with the ends "open" (not folded in the normal way) was superior to the USDA milk-carton trap for trapping H. armigera. The delta trap was therefore used in the following study which compared twelve paired bait combinations with a base of H. armigera pheromone. For placement, traps were suspended on steel stakes in a cotton field so that the trap was at approximately the height of the crop. Traps were placed on rows with a 40m inter-trap spacing. Rows of traps were also separated by 40m. Four complete replicates of the test were established and checked and randomized four times.

Because of the small numbers of H. armigera males captured in this test, for analysis, all readings for each block were pooled (Table 9).

Pheromones for several species, when placed in traps in combinations with H. armigera pheromone, did not significantly reduce trap catch, however, mean separation was poor because of the small numbers of males captured. Only two bait combinations resulted in captures significantly lower than the control treatment (H. armigera bait alone). These were HV and NA baits placed in H. armigera traps. A more extensive field trial was conducted in Australia (see Table 14), and results of this trial give better insight into effect of the various combinations.

Egyptian cotton leaf worm - Spodoptera littoralis:

Trial 10. Numbers of replicates, readings and trap spacing for this test were identical to Trial 9. S. littoralis males were not abundant and very low numbers were captured throughout the test regardless of treatments. These low population levels were probably the result of drought conditions. For analysis, observations were pooled over all readings for each block. The data, however, were insufficient to give good mean separation (Table 10). Essentially no difference was detected in numbers of males captured between most treatments, and further studies should be conducted. Interestingly, one effect noted is that when baits for the red bollworm, D. castanea, are placed in traps baited for S. littoralis, the effect appears to be synergistic, and larger numbers of S. littoralis are captured. Even though the results of this test are not satisfactory in terms of treatment separation, field trials with a closely related species S. litura, which were conducted in Australia, may offer insights into the compatibility of various baits for S. littoralis. Studies with S. litura (Table 16) resulted in better results because of higher populations at the time of testing. Both of these Spodoptera species use slightly different ratios of the same two compounds as attractants. Also, S. litura males, similar to S. littoralis males, appear to be inhibited by both HV and NA pheromone baits therefore, there is some basis for extrapolating results.

False codling moth - Cryptophlebia leucotreta:

Trial 11. Pheromone bait combination studies on the false codling moth (FCM) were conducted in cooperation with the South African Citrus and Subtropical Fruit Research Institute located in Nelspruit, S.A., with the assistance of Drs. P. Newton and M.A. Van den Berg. Results of preliminary tests comparing different trap designs, indicated that the wing-type trap (Pherocon-1C) was superior to four modifications of the

Table 9. Mean number of old world bollworm, Heliothis armigera, males captured in traps baited with H. armigera pheromone alone and in paired combination with various other attractants. Test was performed at the O.T.K. Cooperative Experimental Farm , Groblerstal, South Africa Jan. 24 to Feb. 1, 1984[1/].

Attractant 1	Combination[2/] 2	Trap Type[3/]	$\bar{x}$ number H. armigera captured per block[4/]
HA alb-fib	GM her-lam	or-delta-e.o.	7.3 a
HA alb-fib	ECL zoe-rs	or-delta-e.o.	5.0 a b
HA alb-fib	- - -	or-delta-e.o.	4.0 a b
HA alb-fib	- - -	or-delta-e.c.	3.3 a b c
HA alb-fib	PBW alb-fib	or-delta-e.o	4.3 a b c d
HA alb-fib	CL zoe-rs	or-delta-e.o.	3.5 a b c d
HA alb-fib	HZ alb-fib	or-delta-e.o.	2.3 a b c d
HA alb-fib	HP alb-fib	or-delta-e.o.	2.0 a b c d
HA alb-fib	EI zoe-pc	or-delta-e.o.	2.0 a b c d
HA alb-fib	PS alb-fib	or-delta-e.o.	0.8 b c d
HA alb-fib	FCM alb-fib	or-delta-e.o.	1.5 b c d
HA alb-fib	RB lab-pc	or-delta-e.o.	0.8 b c d
HA alb-fib	HV alb-fib	or-delta-e.o.	0.3 c d
HA alb-fib	NA alb-rs	or-delta-e.o.	0.0 d

1/ Four complete blocks were read and randomized four times for sixteen observations per treatment.

2/ Species codes, manufacturer codes and dispenser types in Appendix 1, 2, and 3, respectively.

3/ Trap type code, in Appendix 4

4/ Means followed by the same letter are not different at the 5% level of significance according to Duncan's Multiple Range Test. Readings for a block were pooled over all blocks for analysis which was performed on [log(males+1)]; actual means are reported here.

Table 10. Mean numbers of Egyptian cotton leafworm, Spodoptera littoralis, males captured in traps baited with S. littoralis pheromone alone and in paired combination with various other attractants. Test was performed at the O.T.K. Cooperative Experimental Farm, Groblerstal, South Africa, Jan. 24, to Feb. 1, 1984[1/].

Attractant Combination[2/] 1	2	Trap Type[3/]	$\bar{x}$ number S. littoralis captured per block[4/]
ECL zoe-rs	RB lab-pc	or-delta-e.o.	6.75 a
ECL zoe-rs	- - -	or-delta-e.o.	2.25 b
ECL zoe-rs	FCM alb-fib	or-delta-e.o.	2.25 b
ECL aoe-rs	PS alb-fib	or-delta-e.o.	2.00 b
ECL zoe-rs	EI zoe-pc	or-delta-e.o.	1.50 b
ECL zoe-rs	GM her-lam	or-delta-e.o.	1.25 b
ECL zoe-rs	- - -	or-delta-e.c.	1.25 b
ECL zoe-rs	HZ alb-fib	or-delta-e.o.	1.00 b
ECL zoe-rs	CL zoe-rs	or-delta-e.o.	1.00 b
ECL zoe-rs	HV alb-fib	or-delta-e.o.	0.75 b
ECL zoe-rs	PBW alb-fib	or-delta-e.o.	0.50 b
ECL zoe-rs	NA alb-rs	or-delta-e.o.	0.25 b
ECL zoe-rs	HA alb-fib	or-delta-e.o.	0.00 b
ECL zoe-rs	HP alb-fib	or-delta-e.o.	0.00 b

1/ Four complete blocks were read and randomized four times for sixteen observations per treatment.

2/ Species codes, manufacturer codes, and dispenser types, in Appendix 1, 2, and 3, respectively.

3/ Trap type codes, in Appendix 4

4/ Means followed by the same letter are not different at the 5% level of significance according to Duncan's Multiple Range Test. Analysis performed on transformed data [log(n+1)]; actual mean values are presented here.

Table 11. Mean number of false codling moth, Cryptophlebia leucotreta males captured in traps[1/] baited with C. leucotreta pheromone alone and in paired combinations with various attractants. Test was performed at the Citrus and Subtropical Fruit Research Institute experimental farm and at the Crocodile Valley Estates, Ltd., Nelspruit, South Africa, from Feb. 3, to April 3. 1984[2/].

Attractant Combination[3/] 1	2	$\bar{x}$ number of C. leucotreta males captured/trap/reading[4/]	
FCM alb-fib	HZ alb-fib	8.82	a
FCM alb-fib	GM her-lam	7.13	a
FCM alb-fib	Control	7.56	a
FCM alb-fib	RB lab-pc	5.47	a
FCM alb-fib	PS alb-fib	6.40	a
FCM alb-fib	PBW alb-fib	5.33	a
FCM alb-fib	HA alb-fib	5.02	a
FCM alb-fib	NA alb-rs	2.51	b
FCM alb-fib	HP alb-fib	1.80	b c
FCM alb-fib	HV alb-fib	1.11	b c d
FCM alb-fib	CL zoe-rs	1.16	b c d
FCM alb-fib	ECL zoe-rs	1.16	b c d
FCM alb-fib	CM alb-fib	0.69	c d
FCM alb-fib	EI zoe-pc	0.58	d

1/ Wing-type - Pherocon-1-C traps used for all treatments.

2/ Five complete blocks were read and randomized nine times for 45 observations per treatment.

3/ Species codes, manufacturer codes and dispenser types, in Appendix 1, 2, and 3, respectively.

4/ Means followed by the same letter are not different at the 5% level of significance according to Duncan's Multiple Range Test. Analysis performed on transformed data [log(n+1)]; actual mean values are presented.

USDA delta trap, and also superior to the high-capacity USDA milk carton trap (unmodified). Preliminary tests also indicated that the commercial Albany bait formulation for false codling moth was superior to the Zoecon bait formulation therefore, the Albany bait was chosen for the base treatment in bait combination tests. Neither of these preliminary tests are reported here, however, more complete tests were conducted later in the year by Dr. P. Newton (see Trials 13, 14, and 15) and those are reported.

In all, fourteen treatments were included in the paired bait combination test (i.e. 13 paired combinations and the control). Five replicates of the bait combination test were established: three replicates were placed in the Research Institute's experimental orchards; and two replicates were placed in commercial orchards belonging to the Crocodile Valley Estates Ltd. Traps were placed in lines with an inter-trap spacing of 80m and inter-line spacing of 80m. Traps were hung from the limbs of trees at approximately 1.5m high. All traps were read, and within a block, re-randomized on a weekly basis. A total of nine trap readings were made, or a total of 45 observations were made on each treatment. Results are presented in Table 11.

Combining baits for several species with FCM moth does not significantly reduce FCM male capture. Compatible baits include those for several domestic species: Heliothis zea (HZ), Lymantria dispar (GM) and Pectinophora gossypiella (PBW).

Trial 12a. In a separate field trial, the performance of six trap designs were evaluated. These designs included: the standard USDA delta trap in two colors - orange (Or-delta) and brown (Br-delta); both colors of the USDA delta trap, with the end panels not folded in (Or-delta-e.o. and Br-delta-e.o.); a wing-type trap (P-1C); and a sixth design - a delta-shaped trap manufactured by Hoechst Corp. (Hoe-delta). The Hoechst "biotrap" is formed from a precreased white plastic sheet which, when assembled, measures 300mm long. The two vertical sides of the triangular opening are 90mm, and the horizontal side is 85mm. A waxed cardboard insert, coated with tack trap, is placed in the trap bottom and acts as the capture surface for the trap. Five complete replicates of this test were established in orange orchards on a 35 meter inter-trap spacing. Traps were checked and randomized a total of sixteen times. Because this test was conducted very late in the growing season, male FCM captures were low and captures were pooled for each replicate over all reading dates for statistical analysis. Results of this trial, presented on Table 12, demonstrate that the P-1C and the Hoe-delta are superior trap designs for FCM.

Trial 12b. This test was conducted to verify the findings of Trial 12a on trap design, and to compare two commercial formulations of FCM baits. Four trap designs were included; Or-delta-e.o., Br-delta-e.o., P-1C, and the Hoe-delta. All of the above trap designs were baited with the commercial Albany formulation. A fifth treatment consisted of the P-1C trap with the commercial Zoecon formulation. Five complete replicates of this test were established in orange orchards, and read and randomized seventeen times.

Table 12. Mean number of false codling moth, Cryptophlebia leucotreta males captured in traps of various designs when baited with commercial preparations of pheromone dispensers. Tests were conducted at the Crocodile Valley Estates, Ltd., Nelspruit, S.A.. Trial 13 2/21 - 3/26/84; Trial 14 6/20 - 8/13/84.[1/]

Attractant[2/]	Trap Type[3/]	$\bar{x}$ number of C. leucotreta males captured per trap[4/]	
		Trial 12a	Trial 12b
FCM alb-fib	or-delta-e.c.	0.8 b	-
FCM alb-fib	or-delta-e.o.	1.6 b	17.20 c
FCM alb-fib	br-delta-e.c.	1.4 b	-
FCM alb-fib	br-delta-e.o.	2.4 b	21.20 b c
FCM alb-fib	Hoe-delta	5.6 a	62.40 a
FCM alb-fib	P-1C	4.8 a	29.14 b
FCM zoe-rs	P-1C	-	2.85 d

1/ Trial 12a - five complete replicates were read and randomized sixteen times for eighty observations per treatment.
Trial 12b - Five complete blocks were read and randomized seventeen times for eighty-five observations per treatment.

2/ Species codes, manufacturer codes and dispenser types, in Appendix 1, 2, and 3.

3/ Trap types, in Appendix 4.

4/ Trial 12a - Means followed by the same letter are not different at the 5% level of significance according to Duncan's Multiple Range test. Analysis performed on transformed data [log(n+1)].
Trial 12b - Means followed by the same letter are not different at the 1% level of significance according to Student-Newman-Keuls Multiple Range Test. Analysis performed on transformed data [$\sqrt{n}$]; actual mean values are presented.

It should be noted that not only do the two commercial formulations differ in dispenser type (Albany - hollow fibers, Zoecon - rubber septa), but the Albany formulation contains 5.68mg of a 1:1 mixture of (E)-8 and (Z)-8 Dodecen-1-ol acetate, while the Zoecon lure contains 7.0mg of (E)-7-Dodecen-1-ol acetate. Results of this trial are presented on Table 12.

The Hoechst delta-type trap captured the largest number of males in both Trials 12a and 12b, and significantly more males than any other trap design in Trial 12b. The P-1C design captured the second largest number of males in both trials. Field observations by Dr. P. Newton indicate that both designs are roughly comparable in trap efficiency, but the Hoe-delta is a superior trap because it is more durable and weathers better in long term field use. The false codling moth is active nearly the whole year, although activity in the cool season is decreased. The Hoe-delta trap, because of its plastic construction, is better able to withstand the hot sunny and rainy conditions, while the P-1C trap, which is constructed of waxed paperboard, begins to deteriorate rapidly.

Interestingly, when the end panels of the USDA delta trap (delta-e.o.) are not folded, the entry port opening is nearly the same dimensions as the Hoe-delta (i.e. USDA-delta-e.o. vertical sides of triangular openings = 100m x horizontal side = 95mm), however, the length of the USDA trap is 50mm shorter. Possibly, the catch differences are not related to the trap dimensions, but are influenced by the trap color (i.e. Hoe-delta is white vs. USDA-deltas are orange and brown). Further studies are being conducted to determine the influence of trap color on trap efficiency.

Trial 12b also clearly demonstrates that the Albany pheromone bait dispenser is superior to the Zoecon dispenser. The attractant (E)-7-Dodecen-1-ol acetate was an early and, apparently, incorrect description of the insect's pheromone. In comparison of the two commercial formulations, most of the differences in performance can probably be attributed to the chemical loading, and not the dispenser type.

Trial 13. Hoechst, Albany, and Zoecon bait dispensers were compared in a long term field trial to determine which had superior field life for monitoring FCM populations. The Hoechst dispenser is a short length of rubber tube, which is baited with the same two compounds used in the Albany dispenser (see Trial 12). For these trials, the Hoechst delta-type traps were used for all dispenser treatments. Trial 13 consisted of four separate field tests in which these three dispenser types were tested. The first field test, which was conducted in a navel orange orchard at Crocodile Valley Estates, Nelspruit, began on 4/9/84, and was terminated on 4/27/84. Two parallel lines of eight trap positions each, were established with a 56 meter between-line and between-trap position spacing. Five replicates of each of the three bait treatments were placed in alternating sequence (Treatment 1, 2, 3 - selected randomly). After each reading, traps were moved one position, until 16 readings were completed. The second field test was a continuation and duplicate of the first test. This test was initiated on 5/2/84 and completed on 6/8/84, at the same site and using the same bait dispensers as the first test. Test three represents a duplicate of tests one and two, at a site 100m due south of tests one and two. This test, using fresh bait dispensers, was initiated on 6/13/84. Test four was located in an orange orchard in

Table 13. Mean number of false codling moth Cryptophlebia leucotreta males captured in Heochst biotraps baited with pheromone dispensers from three manufacturers. Tests were conducted in Nelspruit and Citrusdahl, R.S.A.

Dispenser[2/] Type	$\bar{x}$ number of C. leucotreta males captured[1/] Test 1[3/] (4/9-4/27/84)	Test 2[3/] (5/2-6/8/84)	Test 3[3/] (5/7-6/13/84)	Test 4[3/] (4/13-5/9/84)
Albany-fib	42.8 a	31.8 a	16.8 a	28.8 a
Hoechst-rt	10.4 b	22.2 b	8.6 b	16.6 b
Zoecon-rs	1.4 c	0.6 c	0.8 c	3.8 c

1/ Within a test, means followed by the same letter are not different at the 5% level of significance according to Student-Newman-Keuls Multiple Range Test.

2/ Dispenser types, in Appendix 3.

3/ Tests 1, 2, and 3 had 5 complete replicates read and randomized 16 times. In test 4, 5 complete blocks were read and randomized 12 times.

Citrusdahl, Western Cape Province. In this test, five replicates of each dispenser type were randomized within a single grid of trap points (50x50m spacing). Traps were re-randomized after each reading, and a total of twelve readings were completed.

The results of all four tests are presented on Table 13. Overall, the Albany fiber-formulation captured significantly more males than either of the other two formulations. However, Dr. Newton has noted that in Tests 1 and 2, where the same baits were in the field from April 9 to August 8, the Albany baits consistently captured more males (52.6% of total capture) until June 13th, after which traps baited with the Hoechst formulation captured more (39.2% of the total capture after 6/13). Over the whole period, traps baited with Albany and Hoechst, captured nearly equal proportions. For detection of false codling moth introductions, the Albany formulation would appear to provide the best bait dispenser if traps were rebaited every two months.

Australian Studies

Studies in Australia were conducted in cooperation with the Australian Department of Primary Industries (DPI) and Commonwealth Scientific and Industrial Research Organization (C.S.I.R.O.). In Australia, we had planned to conduct paired bait combination trials with five target species: Heliothis armigera (HA), H. punctigera (HP), Spodoptera litura (CL), S. exempta (NA), and Pectinophora scutigera (PS). Studies were completed on all but Spodoptera exempta because they were not abundant enough at this period in the season. Bait combination tests with this species should be completed during the 1985 growing season. Trials with H. armigera, H. punctigera, and S. litura were all conducted near Toowoomba, Queensland, Australia. Preliminary trap design tests indicated that the wing-type (P-1C) trap was superior to the USDA delta trap for capturing all three species.

Old World bollworm - Heliothes armigera:

Trial 14. Traps baited for H. armigera alone, and in combination with baits for other species were placed in a soybean field on lines 40m apart, and with a 40 meter trap spacing. Lines of traps (4 replicates) were checked and randomized daily for five days. For placement, traps were hung on 1/2" wooden dowels so that the trap would be at approximately the crop height.

Results of this trial are presented on Table 14. Of the two commercial formulations tested, traps baited with the Albany formulation (HA alb-fib) captured significantly more males than traps baited with the Hercon formulation (HA her-lam). Results of testing traps baited with combinations of lures indicate that the number of males captured was significantly lower for all but two combinations (i.e. HA + HZ and HA + NA). Results described earlier, with bait combination studies for H. armigera in Africa, although limited, did not indicate that baits for Spodoptera littoralis (ECL) and S. litura (CL) were strong inhibitors for H. armigera males. In the Australian studies, traps baited with pheromone combinations which included either of these two species, usually contained large numbers of S. litura males and hence the sticky surfaces of these traps were usually covered with S. litura males and wing scales. We

Table 14. Mean number of Old world bollworm, Heliothis armigera, males captured in traps[1/] baited with H. armigera pheromone alone and in paired combination with various other attractants. Test was performed at Toowoomba, Queensland, Australia, Feb. 17 to 22, 1984[2/].

Attractant Combination[3/] 1	2	$\bar{x}$ number of H. armigera males captured per trap[4/]
HA alb-fib	Control	4.2 a
HA alb-fib	HZ alb-fib	4.0 a
HA alb-fib	NA alb-rs	3.1 a b
HA alb-fib	EI zoe-pc	2.5 b c
HA her-lam	- - -	2.3 b c
HA alb-fib	GM her-lam	2.2 b c
HA alb-fib	RB lab-pc	2.0 b c d
HA alb-fib	FCM alb-fib	1.75 b c d e
HA alb-fib	HV alb-fib	1.3 c d e
HA alb-fib	PBW alb-fib	1.2 c d e
HA alb-fib	ECL zoe-rs	0.7 d e [5/]
HA alb-fib	PS alb-fib	0.4 e
HA alb-fib	CL zoe-rs	0.4 e [5/]

1/ Wing type Pherocon-1C traps used for all treatments.

2/ Four complete replicates were read and randomized five times for twenty observations per treatment.

3/ Species codes, manufacturer codes, and dispenser types, in Appendix 1, 2, and 3, respectively.

4/ Means followed by the same letter are not different at the 5% level according to Duncan's Multiple Range Test. Analysis performed on transformed data [log(n+1)]; actual mean levels are presented.

5/ Catches of H. armigera were low in these traps because large numbers of Spodoptera litura were captured early in the test, filling the traps before H. armigera flight occurred. Night observations revealled that H. armigera were readily attracted to these mixtures, and were captured if traps were regularly cleared of S. litura.

suspected that H. armigera catches were low, not because of inhibition, but because traps were simply loaded beyond capacity. To test this theory, we established a separate test of three treatments: traps baited with H. armigera pheromone alone, traps baited with S. litura pheromone alone, and traps with paired baits, H. armigera and S. litura. Four replicates of this test were established in a line (40m inter-trap spacing) on a dirt lane between cotton and soybean fields. Traps were checked and cleared hourly, beginning at 8:00pm (dusk) and finishing at 12:00pm, and also the following morning. Captures of S. litura males began with the first observation at 8:00, and traps continued to capture males through the last check. H. armigera males however, were first found in traps at the 12:00pm check. Traps baited with the combination of the two lures and cleared of S. litura males captured Heliothis males later in the night. Although numbers from this test are too small for statistical analysis, it appears that S. litura and S. littoralis pheromones are probably not strong inhibitors for H. armigera males, but that the Spodoptera species, because they begin to fly earlier in the scotophase period, simply load the traps before H. armigera becomes active.

Trial 15. Design of this test was similar to the test design of Trial 14 (i.e. 4 replicates, 40m inter-trap and inter-line spacing) however, because it was late in the field season, male H. punctigera (HP) captures were low and traps were read and randomized at weekly intervals from Feb. 17 to March 16. Because captures were very low in the first week of this test, it was moved from a soybean field nearing maturity to a soybean field with the plants in an earlier stage of maturity for the final three weeks. Traps in this test, similar to Trial 14, were hung from 1/2" wooden dowels. The trap height was adjusted so that the trap entry ports would be at approximately crop height. In all, ten paired bait combinations with baits for H. punctigera were tested. In addition, three commercial preparations of baits for H. punctigera were included in this test. The bait prepared by Hercon is the standard bait used by the Australian Department of Primary Industries.

Even though this test was extended over four weeks, male captures were still low (Table 15). From results however, some combinations of baits can be selected which are not inhibitors of H. punctigera males. Two bait combinations resulted in male captures which were significantly larger than the control (i.e. HP + GM and HP + RB). The only combination that resulted in significantly lower male captures than the control, was HP + HV (tobacco budworm, H. virescens). Although trap captures for the Zoecon formulation averaged less than half the number of males than the best commercial formulation, no statistical differences between the three formulations could be detected.

Cotton leafworm - Spodoptera litura:

Trial 16. Design of this test was similar to Trial 14 (i.e. 40m inter-trap spacing, 4 replicates), however, traps were read and randomized three times. This trial was conducted in a soybean field at the site of both Trials 14 and 15. Traps were also hung on 1/2" wooden dowels, and positioned so that they were at approximately crop height. Two unpaired bait formulations for S. litura (CL) were included in the test - one commercially prepared by Zoecon and the other prepared by us. The

Table 15. Mean number of Australian bollworm, Heliothis punctigera, males captured in traps [1/] baited with H. punctigera pheromone alone and in paired combination with various other attractants. Test was performed at Toowoomba, Queensland, February 17 to March 16, 1984 [2/].

Attractant Combination [3/] 1	2	$\bar{x}$ number of H. punctigera captured per trap [4/]	
HP alb-fib	GM her-lam	7.5	a
HP alb-fib	RB lab-pc	6.1	a b
HP alb-fib	PBW alb-fib	5.2	b c
HP alb-fib	HA alb-fib	4.3	b c
HP alb-fib	PS alb-fib	3.8	c
HP alb-fib	FCM alb-fib	3.8	c d
HP alb-fib	Control	3.6	c d
HP alb-fib	NA alb-fib	3.4	c d
HP alb-fib	HZ alb-fib	3.4	c d
HP her-lan	- - -	3.3	c d
HP alb-fib	EI zoe-pc	2.9	c d
HP zoe-rs	- - -	1.7	d e
HP alb-fib	HV alb-fib	.2	e

1/ Wing type Pherocon-1C traps used for all treatments.

2/ Four complete replicates were read and randomized four times for sixteen observations per treatment.

3/ Species codes, manufacturer codes, and dispenser types, in Appendix 1, 2, and 3, respectively.

4/ Means followed by the same letter are not different at the 5% level of significance according to Duncan's Multiple Range Test. STET performed on transformed data [log (n+1)]; actual mean values are presented.

Table 16. Mean number of Cotton leaf worm Spodoptera litura, males captured in traps [1/] baited with S. litura pheromone alone and in paired combination with various other attractants. Test was performed at Toowoomba, Queensland, Australia, February 18 to 21, 1984 [2/].

Attractant Combination [3/] 1	2	$\bar{x}$ number of S. litura captured per trap per day [4/]
CL zoe-rs	ECL zoe-rs	9.4 a
CL zoe-rs	HA alb-fib	8.6 a b
CL zoe-rs	GM her-lam	8.4 a b
CL zoe-rs	HZ alb-fib	8.4 a.b
CL zoe-rs	RB lab-pc	7.3 a b c
CL zoe-rs	PBW alb-fib	6.8 a b c
CL lab-rs	- - -	6.3 a b c
CL zoe-rs	Control	5.4 b c d e
CL zoe-rs	FCM alb-fib	5.0 c d e
CL zoe-rs	EI zoe-pc	3.5 d e f
CL zoe-rs	PS alb-fib	2.8 e f
CL zoe-rs	HP alb-fib	1.9 f
CL zoe-rs	HV alb-fib	1.4 f
CL zoe-rs	NA alb-fib	.6 f

1/ Wing type Pherocon-1C traps used for all treatments.

2/ Four complete replicates were read and randomized three times for twelve observations per treatment.

3/ Species codes, manufacturer codes, and dispenser types, in Appendix 1, 2, and 3, respectively.

4/ Means followed by the same letter are not different at the 5% level of significance according to Duncan's Multiple Range Test. Analysis performed on transformed data [log (n+1)]; actual mean values are presented.

commercial formulation was used as the base treatment for all twelve bait combinations.

Only three combinations resulted in male catches significantly lower than the control treatment. When baits for H. punctigera (HP), H. virescens (HV), or S. exempta (NA) were paired with CL baits, a significantly smaller number of males were captured. Baits for HP and HV both contain the same two compounds; (Z)-11-Hexadecenal and (Z)-9-Tetradecenal. HP pheromone baits also contain a third compound (Z)-11-Hexadecenyl acetate. Baits for NA do not contain any of the above compounds but do contain as a major component, a related compound (Z)-9-Tetradecen-1-ol acetate plus as a minor component (Z,E)-9,12-Tetradecadien-1-ol acetate.

When pheromone baits for S. littoralis (ECL) were paired in traps baited for CL, significantly more CL males than the control treatment were captured. As mentioned earlier, baits for these two species are composed of the same two chemical compounds but at slightly different ratios. The significantly higher trap catches in this paired treatment is probably the result of simply more of both chemicals being released from the trap, and this may indicate that normal dispenser loading for S. litura is not optimal.

Trial 17. Results of Trial 16 indicated that traps baited with a combination of CL and PS (Pectinophora scutigera) lures, captured significantly fewer males than traps baited with CL pheromone dispensers alone or in combination with PBW Pectinophora gossypiella pheromone dispensers. Loading rates for both Pectinophora spp are similar (5mg), however, baits for P. gossypiella are formulated as a 50:50 mixture of two isomers (Z,E) + (Z,Z)-7,11-hexadecadienyl acetate, whereas baits for P. scutigera are formulated with only the (Z,Z) isomer. We suspected that either the higher level of the (Z,Z) isomer was acting as an inhibitor, or that the presence of the (Z,E) isomer in baits for PBW was somehow mitigating the inhibitory effect of the (Z,Z) isomer. To determine which scenario was responsible for this apparent difference, a separate field trial was established using the same test design as Trial 16 (4 replicates, 40m inter-trap spacing). Because of time constraints, it was only possible to read and randomize the test twice. For this Trial, 3 concentrations (0.1, 1.0, and 5.0mg) of the two isomers, (Z,Z) and (Z,E)-7,11-hexadecadienyl, alone, and in a 50:50 mixture, were prepared and dispensed onto rubber septa. These dispensers were placed in traps in paired combination with CL pheromone dispensers. In addition, traps baited with CL pheromone alone (control) and in paired combination with commercially prepared baits for PBW and PS, were included as treatments. Table 17 presents the results of this study.

Traps in this trial baited with a combination of CL and commercially prepared PS baits again captured significantly fewer males than the control trap treatment. However, as opposed to Trial 16, this combination did not result in significantly fewer captures than the CL and PBW treatment. When our own preparation of PS bait, equivalent to the commercial loading (i.e. 5mg of the Z,Z isomer), were combined in traps with CL baits, the numbers of males captured were not significantly different from the control. Indeed, the only laboratory-prepared bait which appeared to reduce catch was the 5mg loading of the 50:50 mixture of the two isomers, and this was not significantly different from the

Table 17. Mean number of cotton leafworm, Spodoptera litura, males captured in traps [1/] baited with S. litura pheromone dispensers alone and in paired combination with commercially prepared pheromone dispensers for Pectinophora scutigera and P. gossypiella and laboratory prepared dispensers for three concentrations of the two chemical pheromone components, (Z,E) and (Z,Z)-7,11-hexadecadienyl acetates. Test performed at Toowoomba, Queensland, Australia, Feb. 20 to 22, 1984[2/].

Attractant 1	Combination[3/] 2	$\bar{x}$ number of S. litura captured per trap per day[4/]
Cl zoe-rs	Control	8.0 bc
Cl zoe-rs	PS alb-fib (= 5mg ZZ)	3.5 d
CL zoe-rs	PBW alb-fib (= 2.5mg ZZ + ZE)	5.5 cd
CL zoe-rs	0.1mg Z,E	8.5 bc
CL zoe-rs	1.0mg Z,E	9.1 bc
CL zoe-rs	5.0mg Z,E	9.4 bc
CL zoe-rs	0.1mg Z,Z	11.5 a
CL zoe-rs	1.0mg Z,Z	10.6 b
CL zoe-rs	5.0mg Z,Z	8.1 bc
CL zoe-rs	0.1mg Z,E + Z,Z	10.3 b
CL zoe-rs	1.0mg Z,E + Z,Z	10.3 b
CL zoe-rs	5.0mg Z,E + Z,Z	6.1 cd

1/ Wing type Pherocon-1C traps used for all treatments.

2/ Four complete replicates were read and randomized twice for eight observations per treatment.

3/ Species codes, manufacturer codes, and dispenser types, in Appendix 1, 2, and 3, respectively.

4/ Means followed by the same letter are not different at the 5% level of significance according to Duncan's Multiple Range Test. Analysis performed on transformed data [log (n+1)]; actual mean values are presented.

control. We concluded that at standard loading rates, neither of the isomers, alone or in combination, is a strong inhibitor. If there is not inhibition induced by CL pheromone for the alternate target species (i.e. P. scutigera or P. gossypiella) these would be acceptable combinations for use in detection programs. Apparently, the lower trap catches, which resulted when the commercial preparation of PS pheromone was paired with CL pheromone, were peculiar to that preparation - possibly the result of a chemical impurity present in the loading. If it is an impurity, results of the following Trial (Trial 18) did not indicate that it effects the capture of P. scutigera males.

Pink-spotted bollworm - Pectinophora scutigera:

Trial 18. Pheromone studies for this species were conducted at Biloela ca. 350 kilometers north of the sites of the other Australian studies (Trials 14, 15, 16, and 17). Two commercial bait preparations, PS alb-fib and PS zoe-rs for the pink-spotted bollworm, and our own formulation, PS lab-rs, were included in this study. Our formulation consisted of a 5mg loading of (ZZ)-7,11-hexadecadienyl acetate dispensed in n-hexane (100ul) on rubber septa. The formulation used as the base for paired bait combinations was the PS-zoe-rs. In all, twelve combinations of dispensers were tested. In addition, three trap designs were tested; the Pherocon-1C, and the USDA delta trap with the end panels folded in (normal position) and the end panels left unfolded. Plot design included three complete replicates (lines of traps) with a 40 meter inter-trap spacing.

All three PS bait formulations performed equally well in traps (Table 18). Only two of the bait combination treatments resulted in trap catches significantly lower than the control (i.e. HV - H. virescens and PBW - P. gossypiella). The inhibitory effect of PBW baits (specifically the (ZE) isomer of 7,11 hexadecadienyl acetate), on P. scutigera males, has already been documented several times in the literature. Research is currently under way in Australia to use this compound to disrupt mating of P. scutigera with broadcast pheromone applications. Of the three trap designs tested, both the Pherocon-1C and the standard USDA delta trap (end panels folded) captured significantly larger numbers of males than the delta trap with the end panels unfolded.

Numbers of males captured in Phercon-1C traps are high - probably because of simply a larger horizontal sticky surface (ca. 320cm^2). Delta traps, although providing a total of ca. 330cm^2 of catching surface, have only half of this surface in the horizontal plane. Delta traps with the end panels open, captured an average of less than one-half the number of males than the Pherocon-1C traps. Most of the males captured in the delta traps which had the ends open, were on the bottom (horizontal) sticky surface. Delta traps with the end panels folded (in normal position) captured as many males as the Pherocon-1C traps. We believe this was not because more males entered the trap, but because once the sticky surfaces became loaded with wing scales, males had a harder time escaping. Often, when the standard (closed ends) delta traps were opened for inspection, males flew out. Undoubtedly, at times in this test, all trap designs were loaded with males beyond the point where their capture efficiency (efficiency of retaining males entering a trap) was decreased and occasionally, a trap was filled with insects to the point where capture efficiency was near zero (i.e. for one night's catch, some Pherocon-1C and delta closed end

Table 18. Mean number of pink-spotted bollworm, Pectinophora scutigera males captured in traps baited with P. scutigera pheromone alone and in paired combination with various other attractants. Test was performed at Biloela, Queensland, Feb. 28 to Mar. 2, 1984 [1/].

Attractant Combination[2/] 1	2	Trap Type[3/]	$\bar{x}$ number P. scutigera captured per trap [4/]
PS zoe-rs	- - -	P-1C	65.9 a
PS zoe-rs	- - -	or-delta-e.c.	65.2 a
PS zoe-rs	FCM alb-fib	or-delta-e.o.	33.7 b
PS zoe-rs	GM her-lam	or-delta-e.o.	32.4 b c
PS zoe-rs	HZ alb-fib	or-delta-e.o.	31.2 b c
PS lab-rs	- - -	or-delta-e.o.	26.6 b c d
PS alb-fib	- - -	or-delta-e.o.	26.3 b c d
PS zoe-rs	Control	or-delta-e.o.	25.1 b c d
PS zoe-rs	RB lab-pc	or-delta-e.o.	25.0 b c d
PS zoe-rs	HA alb-fib	or-delta-e.o.	24.9 b c d
PS zoe-rs	EI zoe-pc	or-delta-e.o.	20.7 b c d e
PS zoe-rs	NA alb-rs	or-delta-e.o.	19.0 b c d e
PS zoe-rs	ECL zoe-rs	or-delta-e.o.	15.7 c d e f
PS zoe-rs	CL zoe-rs	or-delta-e.o.	15.2 c d e f
PS zoe-rs	HP alb-fib	or-delta-e.o.	11.8 d e f
PS zoe-rs	HV alb-fib	or-delta-e.o.	7.4 e f
PS zoe-rs	PBW alb-fib	or-delta-e.o.	.9 f

1/ Three complete replicates were read and randomized three times for nine observations per treatment.

2/ Species codes, manufacturer codes, and dispenser types, in Appendix 1, 2, and 3, respectively.

3/ Trap type codes, in Appendix 4

4/ Means followed by the same letter are not different at the 5% level of significance according to Duncan's Multiple Range Test. Analysis performed on transformed data [log(n+1)]; actual mean values are presented.

panel traps captured more than 100 males). Because of these high catches, all traps were replaced daily. Male P. scutigera (PS) captures in traps baited for PS and CL (Spodoptera litura) were low (not significantly different from the control), and we suspected this was because of loading by males of both species. Traps baited with combined baits for either PS and CL (Spodoptera litura) or PS and ECL (S. littoralis) generally caught lower numbers of male PS (not significantly different) than control treatment. Both bait combinations however, also captured S. litura males. We suspected the lower numbers of PS males captured was the result of trap loading.

On March 1, 1984, an additional treatment was added to the test to determine if Spodoptera litura (CL) males caught in traps baited with (PS + CL) and (PS + ECL) were loading traps to the point where PS male captures were reduced. This treatment was a combination of PS and CL baits in delta traps with the end panels in the normal closed position. From previous experience, we knew that closing the end panels of delta traps nearly eliminated entry of CL males. Unfortunately for this test, adequate numbers of the base PS bait formulation (PS zoe-rs) were not available, and traps were baited with the PS alb-fib formulations. The three traps with this treatment (PS + CL in delta traps - ends closed) averaged a mean of 13.3 PS males captured and a total of 2 CL males, while the traps baited with PS-zoe + CL-rs bait combination in delta traps with the end panels open, captured an average of 5.6 PS males and a total of 13 CL males. Although PS captures appear to be greater in the closed delta traps treated with PS and CL bait combination, the zoe-rs formulation of PS pheromone, when placed in delta traps (end closed), captured a larger number of males (mean = 50.3). More studies will have to be completed to determine if the pheromone components of CL and ECL baits inhibit captures of PS males. However, if there is inhibition, and simply not trap loading, then the inhibitory effect is slight, and for detection purposes, baits for PS and CL or ECL could safely be combined in the same trap. However, the inhibitory effect of PS pheromone in traps baited for CL will also have to be considered (Table 16).

French Studies

Pheromone bait combinations in traps for three exotic species were studied in France during the 1984 field season. Work on two of these species Lobesia botrana and Cydia funebrana was nearly completed while studies on a third species Adoxophyes orana will require more extensive testing. Cooperating on this project was Dr. P. Atger of the French Institut National de la Recherche Agronomique.

Plum fruit moth - Cydia funebrana:

Trial 19. Pheromone bait combinations for the plum fruit moth (PFM) were tested at the I.N.R.A. experimental farm (Gotheron) near Valence (3 replicates), and at a commercial orchard in Porit de L'Isere (1 replicate). Within each replicate, traps were read and randomized six times. For statistical analysis, trap catches were summed over all readings within a replicate. Thirteen treatments in all were included in this trial. These included: two commercial formulations (PFM alb-rs and PFM zoe-rs) and eleven paired bait combinations with the PFM alb-rs

Table 19. Mean number of plum fruit moth, Cydia funebrana, males captured in traps [1] baited with C. funebrana pheromone alone and in paired combination with various other attractants. Test was performed at Valence and Porit de L'lsere, France 7/25 - 8/3/84.[2]

Attractant Combination[3] 1	2	$\bar{x}$ number of C. funebrana males captured per trap[4]
PFM alb-rs	Control	15.75 a b
PFM alb-rs	CM alb-fib	24.00 a
PFM alb-rs	GP alb-rs	19.50 a b
PFM alb-rs	TAB alb-fib	14.25 a b
PFM alb-rs	GM her-lam	15.00 a b c
PFM alb-rs	LB alb-rs	10.25 b c
PFM alb-rs	PV alb-fib	11.75 b c
PFM alb-rs	ADOX alb-pc	8.75 b c
PFM alb-rs	AV alb-fib	11.00 b c
PFM alb-rs	EA alb-rs	14.50 b c
PFM alb-rs	OFM alb-fib	4.25 c
PFM alb-rs	FCM alb-fib	0.75 d
PFM zoe-rs	- - -	7.25 b c

1/ Wing type Pherocon-1C traps used for all treatments.

2/ Four complete replicates were read and randomized six times for twenty-four observations per treatment.

3/ Species codes, manufacturer codes, and dispenser types in Appendix 1, 2, and 3, respectively.

4/ Means followed by the same letter are not different at the 5% level of significance according to Duncan's Multiple Range Test. Analysis performed on transformed data [log(n+1)]; actual mean values are presented.

dispensers (Table 9). For placement, traps were hung from the branches of fruit trees in the crown at ca. 1.5m in height.

Although there was no significant difference in the numbers of males captured in traps baited with the two commercial formulations, the Albany (PFM alb-rs) formulation (base treatment) trap averaged twice as many male captures. Nine paired bait combination treatments resulted in numbers of males captured not significantly different than the control. Only two lure combinations yielded trap catches significantly smaller than the control: PFM + OFM alb-fib (Oriental fruit moth) and PFM + FCM alb-fib (false codling moth).

Pheromone dispensers for Oriental fruit moth (OFM) are prepared using the same (Z) and (E) isomers of -8-dodecen-1-ol acetate as PFM in a slightly different ratio. OFM dispensers also are formulated with a minor third compound (Z)-8-dodecen-1-ol, and an overall higher loading rate 4.3mg compared to only 0.2mg for PFM dispensers. Without further field tests, it is impossible to determine why OFM baits are inhibitory to PFM males. Possibly, 1) the titer of compounds is too high, 2) the ratio of compounds may be incorrect, or 3) the additional compound is reducing PFM male captures. However, the other bait combination which captured significantly fewer males than the control was PFM + FCM alb-fib. FCM pheromone dispensers are loaded with 5.68mg of an equal mixture of the two isomers: (Z) and (E)-8-dodecen-1-ol acetate.

In general, low numbers of PFM males were captured in this trial and subsequently, mean separation was poor. However, several combinations of baits are apparently not inhibitory to PFM males and are usable for exotic detection programs. A potential problem with detection trapping for PFM is the capture of OFM males. These were found in low numbers in appropriately baited traps in this trial. Although, with fresh, undamaged specimens, wing markings may be used to distinguish the two species. Specimens with their wing scales damaged, will have to be separated using genetalia characteristics.

European grapevine moth - Lobesia botrana:

Trial 20. Studies with Lobesia botrana (LB) were conducted in a commercial wine grape vineyard at Train l'Herimitage. Preliminary testing indicated the wing-type Pherocon-1C trap was superior to the standard USDA delta trap. Also, in an attempt to determine the effect of trap height, we could not detect differences between traps hung at ca. 1/3m and ca. 1m in height. Therefore for this trial, the Pherocon-1C trap was used as the standard trap in the bait combination studies and all traps were hung ca. 1m high. Three complete replicates of the test were established. Within a replicate, traps were placed on a grid (40m x 40m) of trap points and checked and randomized a total of five times. The treatments included: two commercial LB bait formulations, two trap types and nine paired bait combinations (Table 20).

Although the control treatment (LB zoe-rs) captured over twice as many males as the other commercial bait (LB alb-rs), statistical analysis could not detect significant differences between the treatments. The wing-type trap (P-1C) however, captured significantly more males than delta traps baited with similar dispensers. Only three combination treatments (LB +

Table 20. Mean numbers of European grapevine moth, Lobesia botrana, males captured in traps baited with L. botrana pheromone alone and in paired combination with various other attractants. Test was performed at Tain l'Hermitage, France, 7/26 - 8/3/84.[1]

Attractant Combination[2] 1	2	Trap Type[3]	$\bar{x}$ number L. botrana captured per trap[4]
LB zoe-rs	Control	P-1C	20.00 a
LB zoe-rs	CM alb-fib	P-1C	25.00 a
LB zoe-rs	GM her-lam	P-1C	21.67 a
LB aoe-rs	PV alb-fib	P-1C	11.33 a b
LB alb-rs	- - -	P-1C	9.67 a b
LB zoe-rs	EA alb-rs	P-1C	6.00 b c
LB zoe-rs	- - -	or-delta	4.67 b c d
LB zoe-rs	AV alb-fib	P-1C	2.33 c d e
LB zoe-rs	ADOX alb-pc	P-1C	1.00 d e
LB zoe-rs	FCM alb-fib	P-1C	0.67 e
LB zoe-rs	PFM alb-rs	P-1C	0.67 e
LB zoe-rs	OFM alb-fib	P-1C	0.00 e

1/ Three complete replicates were read and randomized five times for fifteen observations per treatment.

2/ Species codes, manufacturer codes, and dispenser types, in Appendix 1, 2, and 3, respectively.

3/ Trap type codes, in Appendix 4

4/ Means followed by the same letter are not different at the 5% level of significance according to Duncan's Multiple Range Test. Analysis performed on transformed data [log(n+1)]; actual mean values are presented.

CM, GM, and PV, respectively, codling moth, gypsy moth, and the grapeberry moth) resulted in male captures not significantly different from the control. Paralobesia viteana (PV) is a domestic pest of grapes and was included because it offered a good candidate for combination trapping in vineyards.

Gypsy moth (GM) trap catches, earlier in this report (Table 1), were shown to be unaffected by the presence of LB pheromone. This combination could be used in wine growing areas where gypsy moth detection trapping is being conducted.

Although results of this test indicate that inclusion of baits for Clysia (=Eupoecilia) ambiguella, European grape berry moth (EA) in traps baited for LB, significantly reduces male LB captures, another study (personal communication, E. Boller, Swiss Federal Research Station) indicates baits for these two species are cross-compatible.

Summer fruit tortrix - Adoxophyes orana:

Trial 21. The final test conducted in France in 1984, was with Adoxophyes orana (ADOX). Only one large apple orchard was available for this test, and therefore, only one replicate of this test was established and completed. Three commercial bait formulations for ADOX, and three trap designs were included in this test. In addition, eleven paired bait combinaions with ADOX lure were tested (see Table 21). Traps were placed in rows within the orchard with a 50m spacing along and between rows. Traps were hung from wires (espalier culture) at approximately 1.5 meters in height. Reading and randomization of all trap treatments was done weekly for a total of eleven readings.

Generally, the numbers of ADOX males captured was low for all treatments. For analysis, each reading date was treated as a replicate. The ADOX trap and bait combination, which resulted in significantly more male captures than any other treatment, was the I.N.R.A. trap and bait, averaging over twice as many males as the control bait-trap combination (ADOX alb-pc, P-1C).

The I.N.R.A. bait formulation for ADOX is 10mg of a 9:1 combination of (Z)-9-tetradecenyl acetate and (Z)-11-tetradecenyl acetate in rubber septa. The Albany (alb-pc) formulation is 2mg of a 9:1 combination of the same two compounds in poly caps. The Albany (alb) formulation is identical to the Zoecon (zoe) formulation except in the size of the poly caps which are used as dispensers. The higher loading rate of the I.N.R.A. formulation and the different release characteristics of the dispensers could easily explain the differences in field performance. The only known difference between the alb and the zoe formulations was the size of the poly caps used for dispensers (alb - small size, zoe - large).

The I.N.R.A. trap, a large delta-shaped trap with removable sticky liners, is used for monitoring a number of French pest species. This trap is of a flexable design (i.e. precreased windows that can be open or closed, depending on the target species) and the design is also not overly restrictive (i.e. species with specific close range pheromone behaviors do not have difficulty in entering the trap). We are currently planning further field tests with this trap and traps of similar designs.

Table 21. Mean numbers of summer fruit tortrix, Adoxophyes orana, males captured in traps baited with A. orana pheromone alone and in paired combination with various other attractants. Test was performed at Loriol, France, 8/2 - 9/27/84.[1]

Attractant 1	Combination[2] 2	Trap Type[3]	$\bar{x}$ number A. orana captured per reading[4]
ADOX INRA-rs	- - -	INRA-delta	4.55 a
ADOX alb-pc	CM alb-fib	P-1C	2.27 b
ADOX alb-pc	- - -	or-delta	1.55 b
ADOX alb-pc	Control	P-1C	1.64 b
ADOX alb-pc	EA alb-rs	P-1C	1.18 b c
ADOX alb-pc	LB zoe-rs	P-1C	1.00 b c d
ADOX alb-pc	PFM alb-rs	P-1C	0.45 c d e
ADOX alb-pc	FCM alb-fib	P-1C	0.45 c d e
ADOX alb-pc	PV alb-fib	P-1C	0.45 c d e
ADOX alb-pc	GP alb-fib	P-1C	0.18 d e
ADOX alb-pc	OFM alb-fib	P-1C	0.18 d e
ADOX alb-pc	GM her-lam	P-1C	0.09 d e
ADOX zoe-pc	- - -	P-1C	0.09 d e
ADOX alb-pc	AV alb-fib	P-1C	0.00 e
ADOX alb-pc	TAB alb-fib	P-1C	0.00 e

1/ One complete replicate was read and randomized eleven times.

2/ Species codes, manufacturer codes, and dispenser types, in Appendix 1, 2, and 3, respectively.

3/ Trap type codes, in Appendix 4

4/ Means followed by the same letter are not different at the 5% level of significance according to Duncan's Multiple Range Test. Analysis performed on transformed data [log(n+1)]; actual mean values are presented.

Only three bait combination treatments (ADOX + CM, EA, or LB) captured as many males as the control treatment. All other treatments appear to inhibit capture of ADOX males. Of the three combinations which did not significantly reduce male captures, the best in terms of compatibility of the host crop is the ADOX + CM (codling moth) combination. ADOX lure however, when placed in codling moth traps significantly reduces male codling moth captures. The other two baits, which did not significantly reduce catch are EA Clysia ambiguella and LB Lobesia botrana, (both are grape pests). ADOX pheromone, in our tests, has also been shown to be inhibitory to LB males (Trial 20) and the effect of ADOX pheromone on EA has yet to be tested. Therefore, to date, we have not found a bait that is compatible with ADOX baits.

A summary of all the test results to date, on twelve target species, is presented on Table 22. By selecting a target species, and reading vertically down a column, the compatability or incompatability of various species' pheromone baits, when paired with the target species' pheromone in the same trap, can be discerned. Values are all normalized such that control traps represent 100. Values higher or lower represent percent of control values that are caught in traps with paired pheromones. Those close to normal capture (100%) are compatible for the target species. Table 22a identifies those combinations which do not negatively affect capture of either target species. It should be noted that this table only presents the results of our work. Some additional information is available in the literature and some information can probably be deduced from our own information. For example, although we did not test S. litura pheromone baits in traps baited for the target P. gossypiella, we did, however, test baits for S. littoralis. Spodoptera litura and S. littoralis baits contain the same two chemical compounds at slightly different ratios. Since baits for S. littoralis did not negatively affect P. gossypiella capture, baits for S. litura probably would not negatively affect capture either, but until these combinations have been tested, we chose not to include any speculation in our data.

These results demonstrate that surveys for two species can be conducted using traps baited appropriately. However, where these compatible bait combinations have not yet been identified, surveys for the exotic species alone, using a trap bait for only that species, provides a systematic and economical approach. The economic gain is enhanced where placement of these traps for exotics is intermeshed with existing trapping programs.

Table 22. Number of moths of target species captured in traps baited with the target species pheromone alone and in paired combination with other pheromones. Control traps (baited only with target species pheromone) values were normalized at 100 (that is, there were no synergistic or antagonistic effects).

Paired Pheromones Tested		Target Species Tested																						
		ADOX	AV	EA	FCM	PFM	RB	EI	EP	OFM	GP	HA[1/]	HP	HV	HZ	CM	LB	GM	PV	PBW	PS	TAB	ECL	CL
Adoxophyes orana, Summer fruit tortrix	ADOX	100				56										3	5	14		3				
Argyrotaenia velutinana, Red banded leaf roller	AV	0				70											12							
Clysia ambiguella, European grape berry	EA	72				92											30	62						
Cryptophlebia leucotreta, False codling moth	FCM	27			100	5						42	106			3	3	46		64	134		100	93
Cydia funebrana, Plum fruit moth	PFM	27				100										28	3	121		97				
Diparopsis castanea, Red bollworm	RB				72							48	169										300	135
Earias insulana, Spiny bollworm	EI				8							60	81								83		67	85
Epiphyas postvittana, Light brown apple moth	EP																	54						
Grapholita molesta, Oriental fruit moth	OFM	11				27											0							
Grapholita prunivora, Lesser apple worm	GP	11				124																		
Heliothis armigera, Old World bollworm	HA				66							100	119					15		44	99		0	159
Heliothis punctigera, Australian bollworm	HP				24								100					28		5	47		0	35
Heliothis virescens, Cotton bollworm	HV				15							31	6					8		21	30		33	26
Heliothis zea, Corn earworm	HZ				117							95	94					72		107	124		44	156
Laspyresia pommonella, Codling moth	CM	138			9	152										100	125	122		117				
Lobesia botrana, European grapevine moth	LB	61				65										69	100	90		91				
Lymantria dispar, Gypsy moth	GM	6			94	95						52	208			56	108	100		107	129		56	156
Paralobesia viteana, Grape berry moth	PV	27				75											57							
Pectinophora gossypiella, Pink bollworm	PBW				71							29	144					80		100	4		22	126
Pectinophora scutigera, Pink-spotted bollworm	PS				85							10	106								100		89	52
Platynota idaeusalis, Tufted apple budmoth	TAB	0				91										21								
Spodoptera exempta, Nutgrass armyworm	NA				33							74	94					79			76		11	11
Spodoptera littoralis, Egyptian cotton leafworm	ECL				15							17						37		80	63		100	174
Spodoptera litura, Cotton leafworm	CL				15							10									61		44	100

1/ Low HA capture rates with ECL and CL pheromone are the result of traps loading with CL earlier in the night before HA flight commenced. When traps were regularly cleared of CL, HA capture rate was normal.

Table 22a. This matrix summarizes the pheromones that are compatible when used together in the same trap. Shaded cells identify pairs of target insects for which traps can be baited without compromising the effectiveness of either attractant. Other designations summarize results of combinations which have been tested. Slashes through A or B indicate catch of that species is reduced by the mixture. No slash means that target species catch is unaffected by the other species attractant.

Target Species A

Target Species B	ADOX	AV	EA	FCM	PFM	RB	EI	EP	OFM	GP	HA	HP	HV	HZ	CM	LB	GM	PV	PBW	PS	TAB	NA	ECL	CL
ADOX																								
AV	A																							
EA	A																							
FCM	A																							
PFM	A B	B	B	B																				
RB				A																				
EI				A																				
EP																								
OPM	A				A																			
GP	A				A																			
HA				A B		B	B																	
HP				A B		B	B				B													
HV				A							A	A												
HZ				A							A	A												
CM	A B			A B																				
LB	A B	B	B	B	A B				B															
GM	A B		B	A B				B			A B	A B	B	B										
PV	A				A											A								
PBW	B			A B	B						A B	A B	B	B	B	B								
PS						B	B				A B		B	B	B		B							
TAB	A				A										A									
NA				A							A	A					A			A				
ECL				A							A						A			A				
CL				A B		B	B					B	B	B			B					B		

National Pheromone Trap Use - 1984

At the initiation of the exotic pest detection program, little information on the use of pheromone traps nationally was known. Information about the species of insect being trapped, numbers of traps involved and the agencies or individuals responsible for the trapping would be helpful in determining where surveys for exotic pests could be integrated into existing trapping programs, and show where the numbers of traps placed for a species were large enough to warrant combination pheromone testing. To gather this information, all fifty states were surveyed through the area survey coordinators. The following is a summary of that survey. A total of 691,321 traps for various species were reported to be placed in the 48 states that responded. This number included traps placed for 68 different species (Table 23). These species included twenty families in five orders (Table 24). By far, the families Tortricidae, with nineteen species trapped, and Noctuidae, with fourteen species trapped, were the most heavily surveyed families. However, the species trapped over the widest geographic area was the gypsy moth. Thirty-nine states had a total of 212,473 traps placed for gypsy moth. More traps, however, were placed for the boll weevil with a total in 1984, of 276,901. Although these programs, because of the number of traps involved, offer good possibilities for integration of exotic pest trapping programs, the survey results also identified some other insects which are trapped over a wide geographic area, with lower numbers of traps. These species may offer equally good opportunities for integration.

1984 Pilot Scale Exotic Pest Detection Program

Included in Appendix 5 and Appendix 5a of this report are the 1984 exotic pest detection survey recommendations and a summary of the 1984 pilot scale exotic pest trapping program.

Table 23. Number of traps placed for each species of insect which was surveyed for using attractants - 1984.

SCIENTIFIC NAME	COMMON NAME	NO. TRAPS
Agrotis ipsilon	Black cutworm	701
Agrotis orthogonia	Pale western cutworm	100
Amphamallon majalis	European chafer	25
Anarsia lineatella	Peach twig borer	48
Anastrepha ludens	Mexican fruit fly	4,825
Anthonomus grandis	Boll weevil	276,901
Anticarsia gemmatalis	Velvetbean caterpillar	9
Aonidiella aurantii	California red scale	207
Archips argyrospilus	Fruittree leafroller	15
Argyrotaenia citrana	Orange tortrix	30
Argyrotaenia velutinana	Redbanded leafroller	111
Autographa californica	Alfalfa looper	50
Ceratitis captiata	Med fly	55,496
Ceratitis rosa	Natal fruit fly	8,306
Choristoneura conflictana	Large Aspen tortrix	40
Choristoneura fumiferana	Spruce budworm	242
Choristoneura rosaceana	Oblique banded leafroller	1,245
Dacus cucurbitae	Melon fly	18,419
Dacus dorsalis	Oriental fruit fly	18,776
Dacus tryoni	Queensland fruit fly	8,309
Dendroctonus rufipennis	Spruce bark beetle	25
Diabrotica virgifera	Western corn rootworm	109
Dioryctria disclusa	Coneworm	20
Eucosma sonomana	Western pine shoot borer	200
Euxoa auxiliaris	Army cutworm	150
Euxoa ochrogaster	Redbacked cutworm	100
Grapholitha molesta	Oriental fruit moth	67
Grapholitha prunivora	Lesser appleworm	31
Heliothis virescens	Tobacco budworm	248
Heliothis zea	Corn earworm/Cotton bullworm	502
Hoplocampa testudinea	European apple sawfly	10
Hylemya brassicae	Cabbage maggot	4
Laspeyresia pomonella	Codling moth	2,144
Lymantria dispar	Gypsy moth	212,473
Oberea tripunctata	Dogwood twig borer	0
Orgyia pseudotsugata	Douglas-fir tussock moth	3,425
Orthosia hibisci	Speckled green fruitworm	57
Ostrinia nubilalis	European corn borer	926
Pandemis limitata	Three-lined leafroller	2
Papillia japonica	Japanese beetle	11,487
Paralobesia viteana	Grape berry moth	6
Pectinophora gossypeilla	Pink bollworm	30,080
Phyllonorycter blancardella	Spotted tentiform leafminer	118
Platynota flavedana	Variegated leafroller	20
Platynota idaeusalis	Tufted apple budmoth	40
Plutella xylostella	Diamondback moth	11
Podossesia syringae	Lilac/Ash borer	18
Prionoxystus robinae	Carpenter worm	15
Pseudaletia unipuncta	True armyworm	11
Pseudococcus comstocki	Comstock mealy bug	240

Table 23 (continued).

SCIENTIFIC NAME	COMMON NAME	NO. TRAPS
Pseudoplusia includens	Soybean looper	19
Quadraspidiotus perniciosus	San Jose scale	280
Rhagoletis angulata	Cherry fruit fly	3,670
Rhagoletis mendax	Blueberry maggot	0
Rhagoletis pomonella	Apple maggot	22,049
Rhyacionia buoliana	European pine shoot moth	1,306
Rhyacionia frustrana	Nantucket pine tip moth	173
Rhyacionia neomexicana	Tip moth	24
Schizaphis graminum	Greenbug	15
Sparganothis sulfureana	Sparganothis fruitworm	10
Spilonota ocellana	Eyespotted budmoth	15
Spodoptera exigua	Beet armyworm	25
Spodoptera frugiperda	Fall armyworm	264
Synanthedon exitiosa	Peach tree borer	114
Synanthedon pictipes	Lesser peach tree borer	84
Tischeria malifoliella	Apple trumpet leafminer	2
Trichoplusia ni	Cabbage looper	77
Trogoderma granarium	Khapra beetle	6,800

Table 24. National summary of the orders and families of insects for which pheromone traps were placed - 1984.

ORDER	FAMILY	NO. SPECIES
Lepidoptera	Tortricidae	19
Lepidoptera	Noctuidae	14
Diptera	Tephritidae	9
Lepidoptera	Sesiidae	3
Lepidoptera	Pyralidae	2
Lepidoptera	Lymantriidae	2
Lepidoptera	Gelechiidae	2
Homoptera	Diaspididae	2
Coleoptera	Scarabaeidae	2
Lepidoptera	Tischeriidae	1
Lepidoptera	Plutellidae	1
Lepidoptera	Gracillariidaa	1
Lepidoptera	Cossidae	1
Hymenoptera	Tenthredinidae	1
Homoptera	Pseudococcidae	1
Homoptera	Aphididae	1
Diptera	Anthomyiidae	1
Coleoptera	Scolytidae	1
Coleoptera	Dermestidae	1
Coleoptera	Curculionidae	1
Coleoptera	Chrysomelidae	1
Coleoptera	Cerambycidae	1

Appendix 1. Species codes - scientific names - common names.

ADOX	*Adoxophyes orana*	summer fruit tortrix
AV	*Argyrotaenia velutinana*	red banded leafroller
CL	*Spodoptera litura*	cotton leafworm
CM	*Laspeyresia pommonella*	codling moth
EA	*Clysia (Eupoecilia) ambiguella*	European grape berry moth
ECL	*Spodoptera littoralis*	Egyptian cotton leafworm
EI	*Earias insulana*	spiny bollworm
EP	*Epiphyas postvittana*	light-brown apple moth
FCM	*Cryptophlebia leucotreta*	false codling moth
GM	*Lymantria dispar*	gypsy moth
GP	*Grapholita prunivora*	lesser apple worm
HA	*Heliothis armigera*	Old World bollworm
HP	*Heliothis punctigera*	Australian bollworm
HV	*Heliothis virescens*	tobacco budworm
HZ	*Heliothis zea*	corn earworm
LB	*Lobesia botrana*	European grapevine moth
NA	*Spodoptera exempta*	nutgrass armyworm
OFM	*Grapholita molesta*	Oriental fruit moth
PBW	*Pectinophora gossypiella*	pink bollworm
PFM	*Cydia funebrana*	plum fruit moth
PS	*Pectinophora scutigera*	pink-spotted bollworm
PV	*Paralobesia viteana*	grape berry moth
RB	*Diparopsis castanea*	red bollworm
TAB	*Platynota idaeusalis*	tufted apple bud moth

Appendix 2. Manufacturer codes:

alb	Albany International
her	Health-Chem Corporation
lab	prepared by Otis Methods Development Center
zoe	Zoecon Corporation

Appendix 3. Dispenser type codes:

lam	3 layer plastic laminate
fib	hollow micro fibers
rs	rubber septa
pc	poly caps
rt	rubber tube

Appendix 4. Trap codes:

M.C. — high capacity USDA gypsy moth trap (milk carton)

delta — USDA delta trap

Prefixes:

or = orange

br = brown

Suffixes:

e.o. = the end panels of trap not folded in (open)

e.c. = the end panels folded in normal position (closed)

P-1C — Pherocon-1C "wing type" trap

Hoe-delta — delta type "biotrap" manufactured by Heochst

I.N.R.A.-delta — large delta trap used by I.N.R.A.

Appendix 5.

Exotic Pest Detection

1984 Survey Recommendations

Otis Methods Development Center

EXOTIC PEST DETECTION SURVEY RECOMMENDATIONS

for

Adoxophyes orana	Summer fruit tortrix moth
Cryptophlebia leucotreta	False Codling moth
Cydia funebrana	Plum fruit moth
Lobesia botrana	European grape vine moth
Rhagoletis cerasi	European cherry fruit fly
Spodoptera littoralis	Egyptian cotton leafworm
Spodoptera litura	African cotton leafworm

November 29, 1984

GENERAL TRAPPING GUIDELINES

Careful preparation and handling of traps and baits is an important part of conducting a productive detection survey. Traps should be assembled according to instruction provided, giving particular attention to critical dimensions (e.g. entry port openings). Damaged traps that have tack-trap on the outside surfaces or do not have enough tack-trap on the inside (catching surfaces) should be discarded or returned. Baits (pheromone dispensers) should be handled carefully to prevent contamination of the outside trap surfaces or cross contamination with other baits. Careful handling of baits with forceps or disposable rubber gloves should prevent contamination of the trapper and the traps. Forceps should be cleaned and gloves should be changed when switching bait types. Preparing all trap components first, and subsequently baiting these traps with lures, should also minimize contamination of the outer trap surfaces.

Baits should be placed in traps so that when traps are serviced the bait can easily be moved to a new trap or, in the case of some trap types (wing-type traps), the bait should be attached to the portion of the trap that does not require replacement (i.e. underside of the roof of wing-type traps). Under no circumstances should baits be placed in the adhesive (i.e. tack-trap). Placement of polycap type bait dispensers (small plastic vials) is facilitated by wrapping a piece of thin copper wire around the hinge of the dispenser. The trailing ends of the wire can then be stapled to the trap interior. Do not open polycap dispensers. Other pheromone dispenser types (rubber septa, hollow fibers, and laminates) can simply be stapled centrally in the trap.

Unused traps should be stored in a cool, dry area that is free of pheromone dispensers. Baits should be stored in tightly sealed glass containers in a freezer. Again, care should be exercised so that different bait types are not mixed in the same container (which would result in cross contamination). Bait dispensers and containers of baits should not be exposed to strong light for long periods of time. Some pheromone components are photosensitive and will degrade rapidly if left in bright light.

Traps should be serviced as often as possible. Frequent checking will prevent trap loading and will facilitate identification of trapped specimens. It is suggested that traps be checked at least every two weeks (at a minimum) unless other conditions suggest more frequent checking is necessary (i.e. traps overload or replacement of pheromone dispensers is required). Guidelines for handling collected specimens is covered in a separate section of these recommendations, Appendix 1. Volatility and degradation rates vary between pheromone components among the various species, and release characteristics are different for the different types of dispensers. For these reasons, no generalizations can be made about field life of baits. The expected field life and recommended intervals for bait replacement are listed for each individual species.

Placement of traps (i.e. height, crop) is also outlined for each individual species. Flight characteristics and responses to pheromones vary from species to species; closely following these recommendations will maximize trap efficency. Little or, in most cases, no work has been done on the optimal trap spacing for detection of these exotic pests. However, information is available about flight ability and response to pheromone over distances.

Given this information, the best approach is to place traps over as large a geographic area as feasible while optimizing the location of each individual trap. Where possible, traps should be placed within the host crop of the target insect. Crops which are not sprayed will likely harbor larger populations of the target species and placement at these sites will enhance detection. When trapping two species with the same trap, placement of the trap where host plants are adjacent or in close proximity will be the most desirable location. Recommendations for combinations are listed under each individual species. When traps are hung within a crop, care should be exercised so that entry ports are not blocked by vegetation or, in some cases, blocked by the stake the trap is hung on. When servicing traps, any damaged traps, or traps that have the sticky surface saturated with insects, dirt or debris, should be replaced.

Adoxophyes orana Summer fruit tortrix moth

Hosts: Apple, Pear

Distribution: See map

Biology: Adoxophyes orana is a bivoltine tortricid which overwinters as 2nd or 3rd instar larvae in bud axiles, bark crevices and under dry leaves. Diapause is induced by short (less than 12-hour) day length. In the Netherlands, diapause is normally initiated in late September or early October. Diapausing larvae can survive the low winter temperature of Northern Europe.

Overwintering larvae begin feeding in the spring after an accumulation of approximately 67 degree-days C (ddC) (in Romania) based on a 9°C developmental threshold. These larvae feed on the leaves and flowers of the host plants and pupate in May. In France, adult moths emerge during the first part of June, with oviposition shortly thereafter. Second generation adults emerge in mid-August. Flight occurs at temperatures above 13°C. The summer generation of larvae lasts, on average, 430 ddC above a threshold of 7°C in France, and feed mainly on the leaves. Second generation larvae feed on fruit before entering diapause in the fall.

Up to 10% and 20% fruit loss has occurred in France and Germany, respectively.

Potential U.S. distribution: Throughout the US, wherever host plants occur (see map).

Recommended survey areas: Major apple and pear producing areas (see map). WA, NY, MI, CA, PA, VA, NC, WV, OR, NJ, IL, MA, ME, ID, CO, MD, OH, MO, NH, WI, IN, UT, VT, CT

Pheromone: 90:10 mixture of (Z)-9:(Z)-11-tetradecenal acetate
dispenser type - polycap
field life - 3 months, if a longer trapping period than 3 months is anticipated, replace baits at midseason.

Commercial source of pheromone dispensers: Pest-Select International, Inc. Corporation

Traps: USDA - delta trap - ends open (i.e. the ends of the trap which are normally folded in to form a small triangular entry port, should not be folded)

Trap placement: Within apple or pear orchards, suspended from the limbs of trees ca. 1.5 m in height.

Recommended combinations: None presently recommended.

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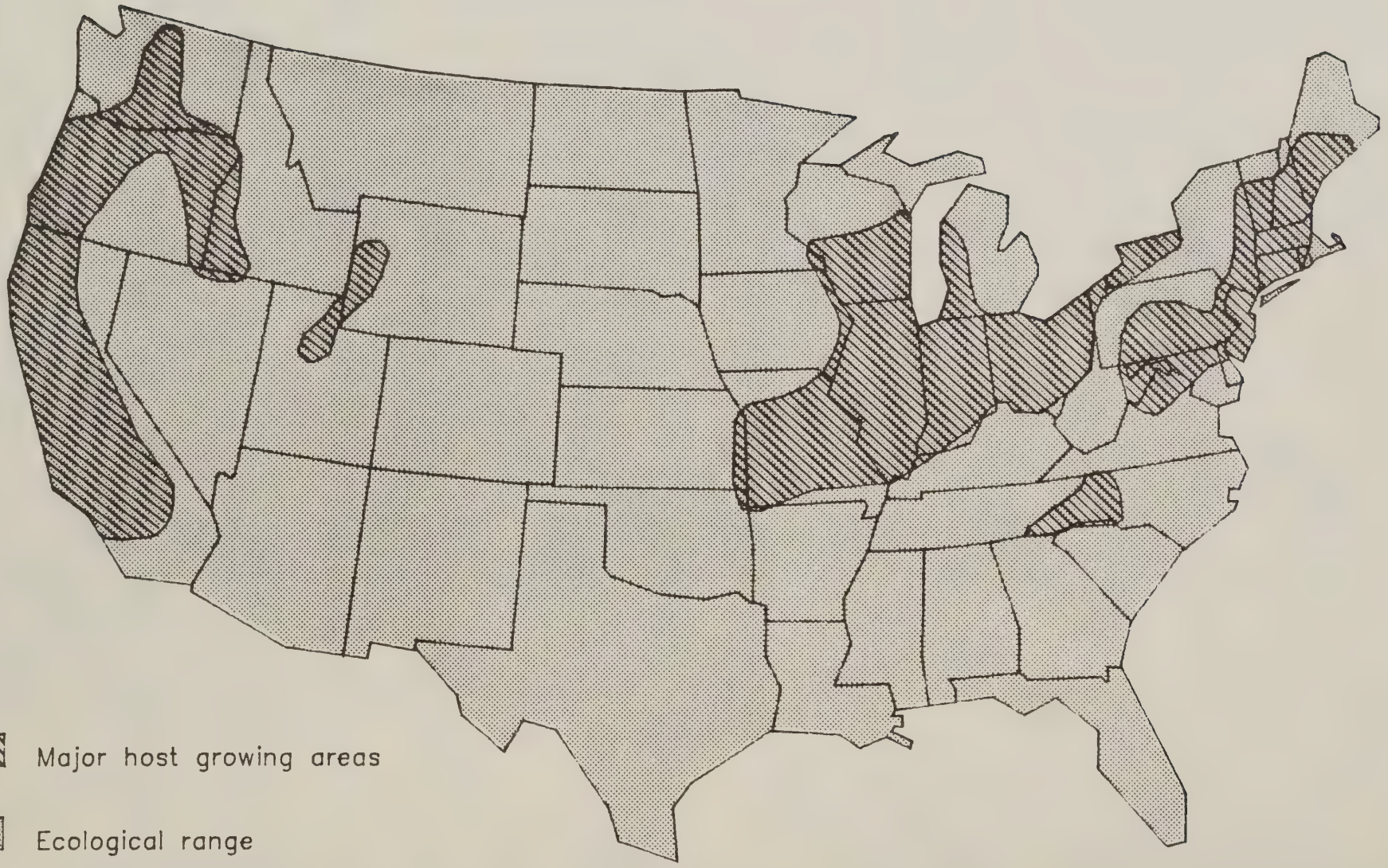
Adoxophyes orana

World distribution

Adoxophyes orana



EXOTIC PEST DETECTION SURVEY RECOMMENDATIONS

Cryptophlebia leucotreta False codling moth

Hosts: Citrus, Cotton, Sorgum, Corn, Peach, Oak, etc.

Distribution: See map

Biology: This tortricid is multivoltine with up to six generations annually in S. Africa, where it breeds throughout the year on oranges. Depending on temperature, it can complete a generation in 45-100 days. There was no mention of a diapause in the literature reviewed. Females fly at night and usually deposit about 150-200 eggs, beginning 2-3 days after emergence. Eggs are laid on the leaves and bolls of cotton and on the fruit of citrus, and hatch in 4-14 days. Eight days of temperatures below 1.1°C is lethal to the eggs, however high mortality will also occur at 13°C and 30 percent relative humidity. The developmental threshold for eggs is 11.9°C. Larvae feed in the fruit and bolls and then drop to the soil surface to pupate. Twenty-one days of temperatures below -0.6° is lethal to larvae, and prepupal and pupal mortality is high at an average ambient temperatures of 10.5°C and below.

Although this species has a wide host range, apparently it is of greatest economic importance on citrus and cotton, which have suffered major losses in Africa.

Potential U.S. Distribution: Where the average annual minimal temperatures are above 10°C (see map).

Recommended survey area: Major citrus and cotton growing areas (see map). TX, CA, MS, AZ, AR, LA, OK, AL, TN, MO, NM, SC, GA, NC, FL

Pheromone: 50:50 mixture of (Z):(E)-8-dodecenyl acetate
dispenser type - strips of hollow fibers
field life - 8 weeks, replace bait midseason or every 8 weeks, which ever time period is shorter

Commercial source of pheromone dispensers: Pest-Select International Inc.

Traps: Pest-Select International Inc. (Wing trap); Zoecon Corporation[1/] (Pherocon-1-C)

Trap placement: In citrus and peach orchards traps should be suspended from the tree limbs at ca. 1.5 meters in height. In row crops, traps should be placed on stakes at the same height as the crop.

Recommended combinations: None presently recommended.

1/ T.R.E.C.E. Corp., distributor of Zoecon Products.

Otis Methods Development Center 11/29/84


160 140 120 100 80 60 40 20 0 20 40 60 80 100 120 140 160 180
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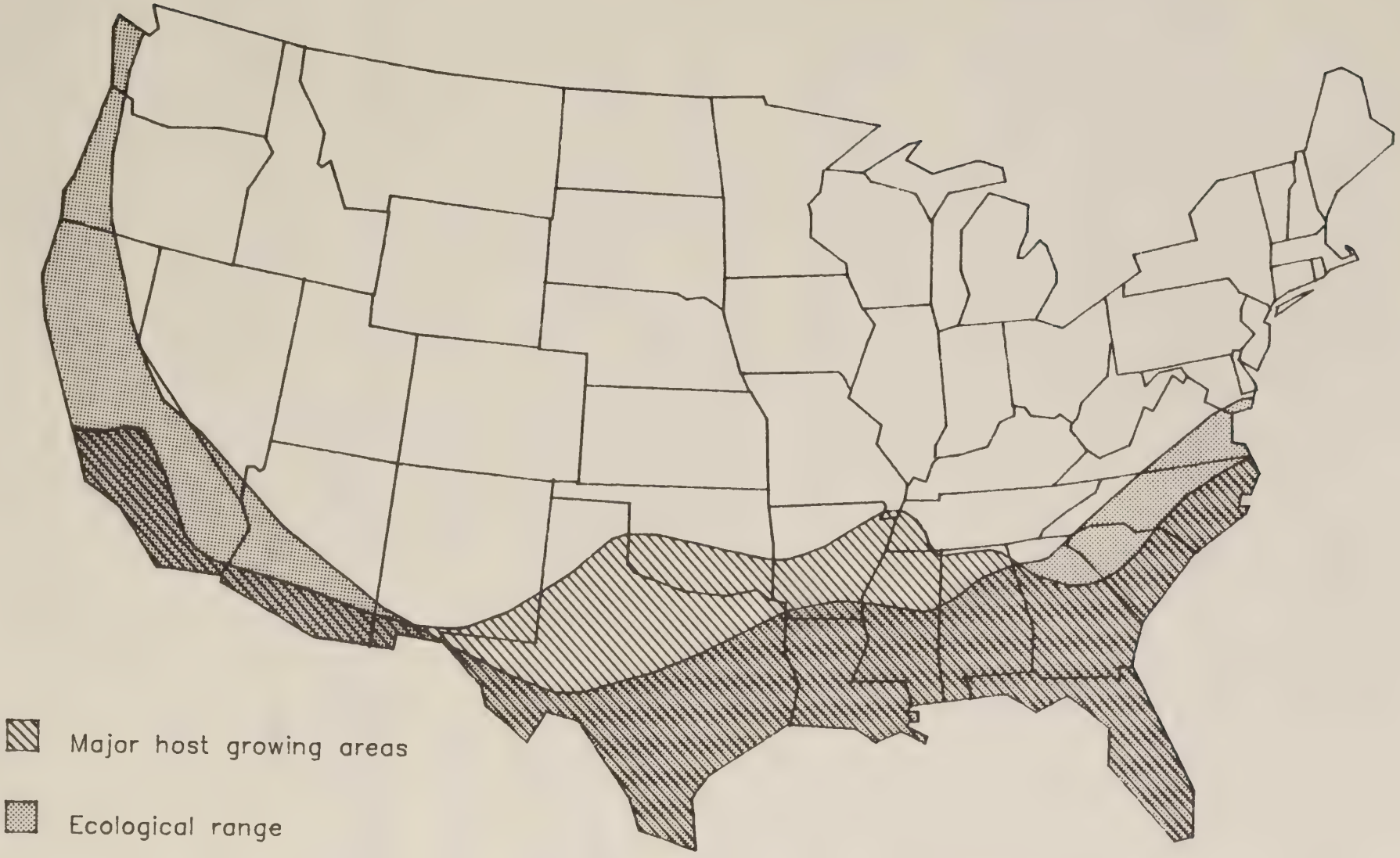
120 100 80 60 40 20 0 20 40 60 80 100 120 140 160 180
Cryptophlebia leucotreta
World distribution

Cryptophlebia leucotreta



Cydia funebrana (=Grapholitha) Plum fruit moth

Hosts: Plum, Cherry, Apple, Peach, Apricot, Pear, Walnut

Distribution: See map

Biology: This tortricid overwinters as prepupae in cocoons under bark flaps. It has a facultative diapause induced in 2nd and 3rd instar larvae by day lengths less than 14 hours, and completes two generations in temperate areas, but three in S.W. Hungary and Iran. Adults emerge in the spring at 30 accumulated degree-days C (ddC), based on a 10°C developmental threshold, with a generation time of 420 ddC. The second generation flight period begins between 450-500 ddC (June-July). Females lay 49-150 eggs singly on leaves or fruit. Larval feeding in fruit causes a characteristic emission of gum, and first generation larvae may cause premature fruit drop. Second generation larvae cause the greatest damage in later fruiting varieties.

Potential U.S. distribution: Throughout the US wherever host plants occur.

Recommended survey areas: Major plum, cherry, apple and peach producing areas (see map). WA, NY, MI, CA, PA, VA, NC, WV, OR, NJ, IL, MA, ME, ID, CO, MD, OH, MO, NH, WI, IN, UT, VT, CT, MT

Pheromone: 95:5 mixture of (Z):(E)-8-dodecenyl acetate
dispenser type - rubber septa
field life - 4 weeks, replace baits midseason or every 4 weeks which ever time period is shorter

Commercial sources of pheromone dispensers: Pest-Select International, Inc.

Traps: Pest-Select International, Inc. (Wing trap); Zoecon Corporation (Pherocon-1-C)

Trap placement: within orchards of host trees, suspended from limbs ca. 1.5m high.

Recommended combinations: Plum fruit moth baits can be combined with no more than one of the following baits: gypsy moth, Lymantria dispar, or codling moth, Laspeyresia (Cydia) pomonella.

Combination #1 - Traps baited for C. funebrana and L. dispar should be located in orchards which are hosts of C. funebrana but near potential hosts for L. dispar. Plum fruit males are relatively weak dispersers compared to gypsy moth males.

Pheromone dispensers for L. dispar should be USDA dispensers (Hercon).

Combination #2 - Traps baited for C. funebrana and L. pomonella should be placed in orchards which both species use as a host or in mixed orchard situations where favored hosts of both species are available. In

the latter case, the trap should be placed in the orchard which is the favored host (i.e. plum) of C. funebrana because these males are relatively weak dispersers compared to L. pomonella males.

Pheromone dispensers for L. pomonella available from Pest-Select International, Inc.

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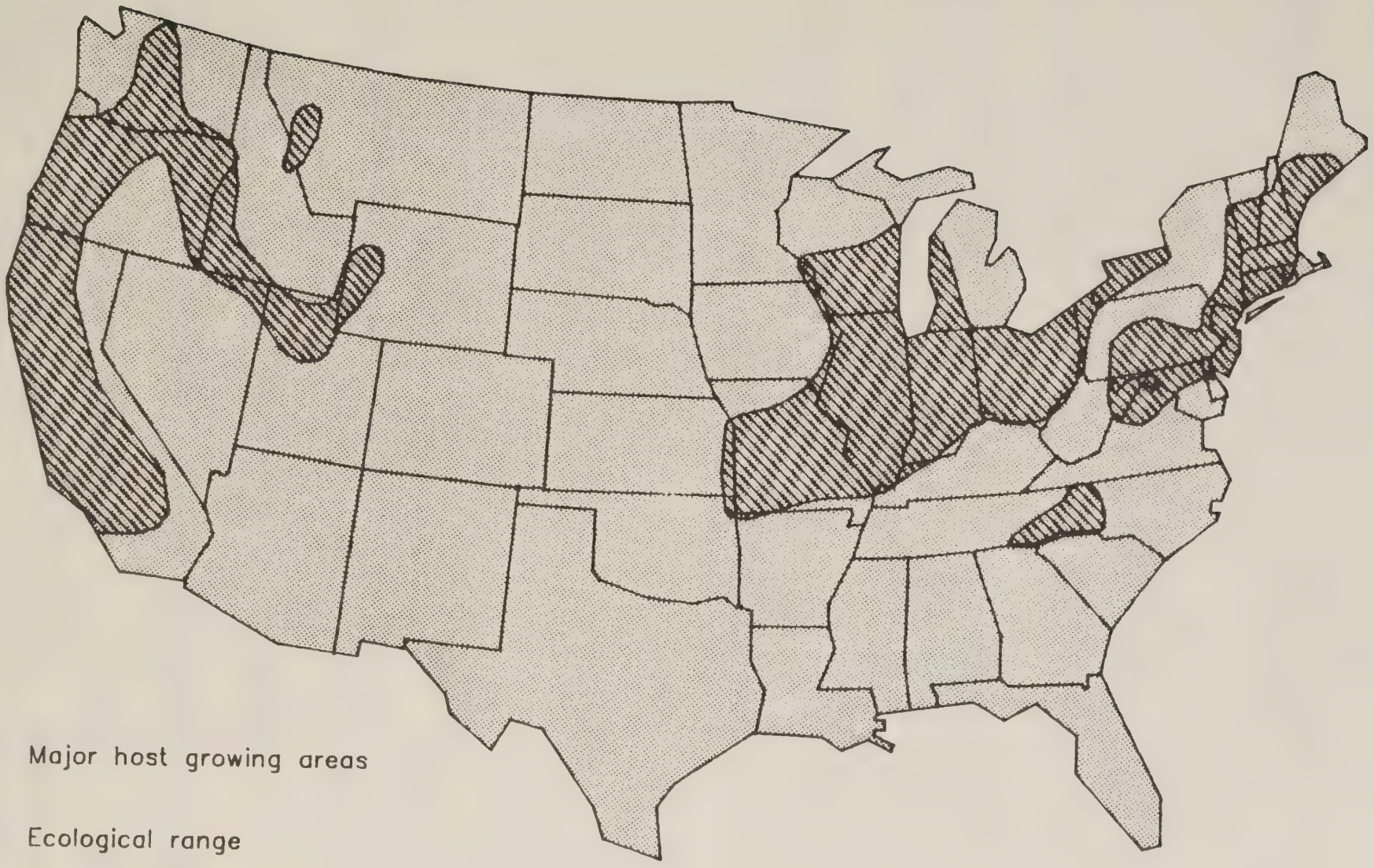
120 100 80 60 40 20 0 20 40 60 80 100 120 140 160 180
Cydia funebrana
World distribution

Cydia funebrana



Lobesia botrana European grape vine moth

Hosts: Grapes (Vitis)

Distribution: See map

Biology: Lobesia botrana is a multivoltine species with four generations per year, depending on latitude. Diapause is facultative, occurring in the pupal stage whenever the eggs or early larval stages are exposed to day-lengths of less than 12 hrs. Over-wintering pupae live within cocoons located under fallen leaves or in cracks in the soil or under the grape vine bark.

Spring adult emergence will begin whenever the daily average air temperature is above the minimal threshold temperature of 10°C for 10-12 days. Traps for monitoring spring adult flight should be set up after 60 degree-days C (ddC). Adults will fly at dusk whenever the temperature is above 12°C, but rainfall or wind will reduce flight.

First generation eggs are laid on the flower buds or pedicels of the vine. The larvae feed on the bud clusters before pupating inside them or under the rolled leaf . It takes an average of 402 ddC to complete the first generation from sexual maturation of the parents to pupation.

The second generation eggs are laid singly on individual grapes. The larva will enter the grape and feed before pupating inside the grape. To complete the second generation, 441 ddC are required.

The third generation larvae also feed on the grapes but, unlike the second generation, will feed on more than one grape. The third generation normally produces diapausing pupae but may also give rise to a partial fourth generation.

Potential U.S. distribution: Throughout the U.S., wherever host plants occur (see map).

Recommended survey area: Major grape producing States (see map). CA, NY, WA, MI, PA, OH, AZ, AR, NC, MO

Pheromone: (E,Z)-7,9-dodecenyl acetate
dispenser type - rubber septa
field life - 3 weeks, replace baits every 3 weeks.

Commercial source of pheromone dispensers: Zoecon Corporation; Pest-Select International, Inc.

Traps: Pest-Select Int., Inc. (Wing trap); Zoecon Corporation (Pherocon-1-C).

Trap placement: Lobesia botrana males are weak dispersers; therefore traps should be placed within grape vineyards. Trap should be suspended from wires or vines ca. 1/2 to 1 m above the ground. Care should be exercised in trap placement so that grape foliage does not block trap entry ports.

Recommended combinations: Compatible pheromones include attractants for the gypsy moth, Lymantria dispar, and the codling moth, Laspeyresia (Cydia) pomonella. Only one of these attractants should be combined at a time in traps baited for Lobesia botrana.

Combination #1 — Traps baited for L. botrana and L. dispar should be placed in vineyards located close (within 300 m) to hosts for the gypsy moth.

Pheromone dispensers for L. dispar should be USDA dispensers (Hercon).

Combination #2 — Traps baited for L. botrana and L. pomonella should be placed in vineyards located adjacent to host (apple, pear, etc.) for the codling moth. In considering this combination, the effect of monitoring codling moth population with traps placed outside of the host crop will have to be weighed against the objective of the monitoring program (i.e. timing spray application, etc.).

Pheromone dispensers for L. pomonella available from Pest-Select Int., Inc.

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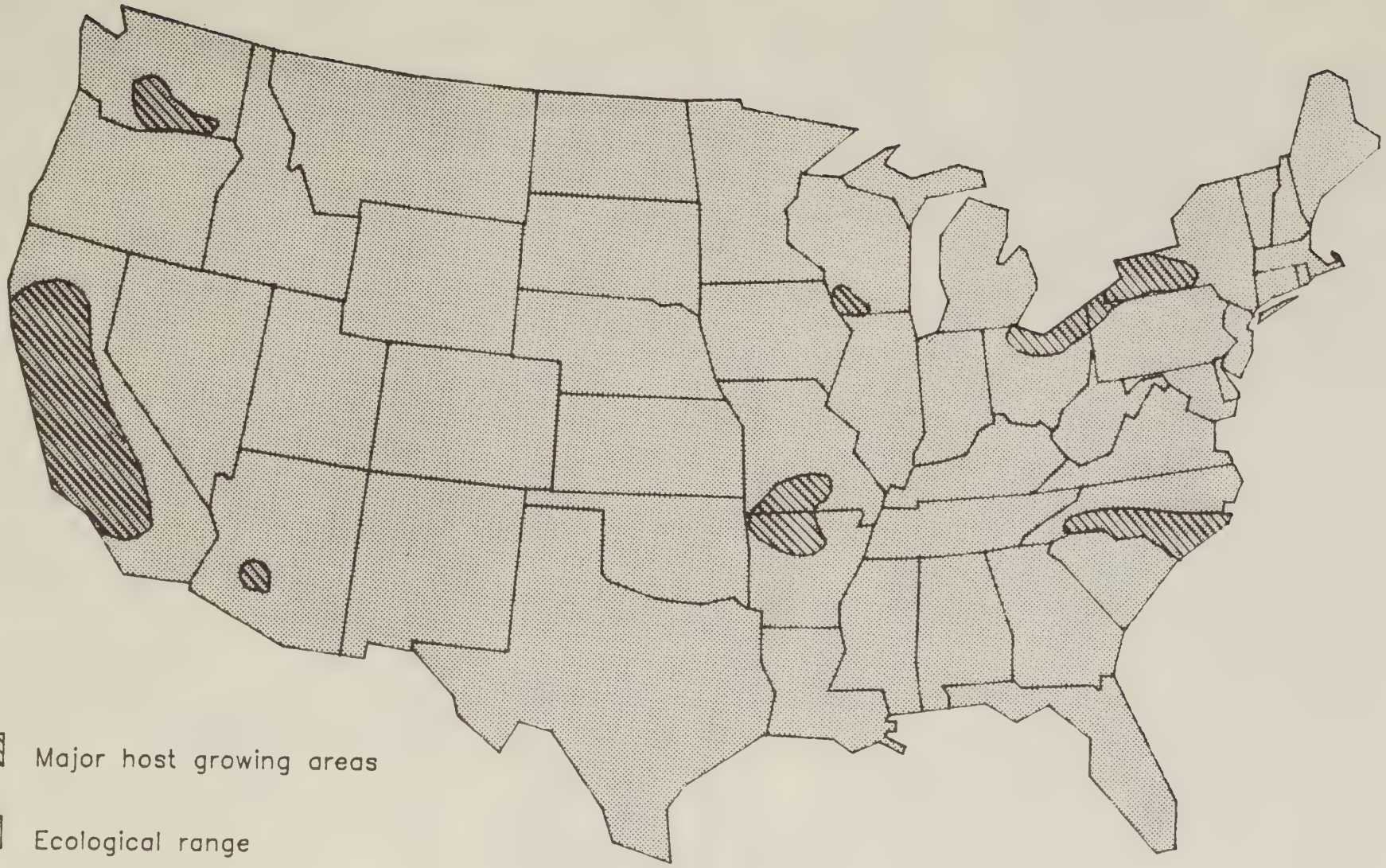
Lobesia botrana

World distribution

Lobesia botrana



Rhagoletis cerasi European cherry fruit fly

Hosts: Cherry, Lonicera

Distribution: See map

Biology: This fruit fly has one generation per year and overwinters as a puparium in the soil. In Switzerland, adult emergence occurs in the spring, after 430 degree-days C have accumulated at a soil depth of 5 cm, based on a 5°C developmental threshold,. This usually occurs in May or June, with the flight period lasting from one to two months. Puparia require cold soil temperatures (less than 0°C), for at least one month, for the majority to break diapause. Eggs are laid in the fruit where the larvae feed for 13-30 days. Damage can be as severe as in Italy, where up to 90 percent of the fruit has been infested.

Potential U.S. distribution: Throughout the U.S., wherever host plants occur (see map).

Recommended survey area: Major cherry producing States (see map). MI, WA, OR, CA, NY, PA, UT, WI, MT, CO, ID

Attractant: Ammonium acetate or Ammonium carbonate

Commercial sources of attractant dispensers: Zoecon Corporation

Traps: Colored sticky panels from Great Lakes IPM Corporation; Swiss Federal Research Station

Recommended combinations: Because the attractants (visual and olfactory) employed by this trap are used by a variety of fruit pests, monitoring of native species distribution and abundance is also possible.

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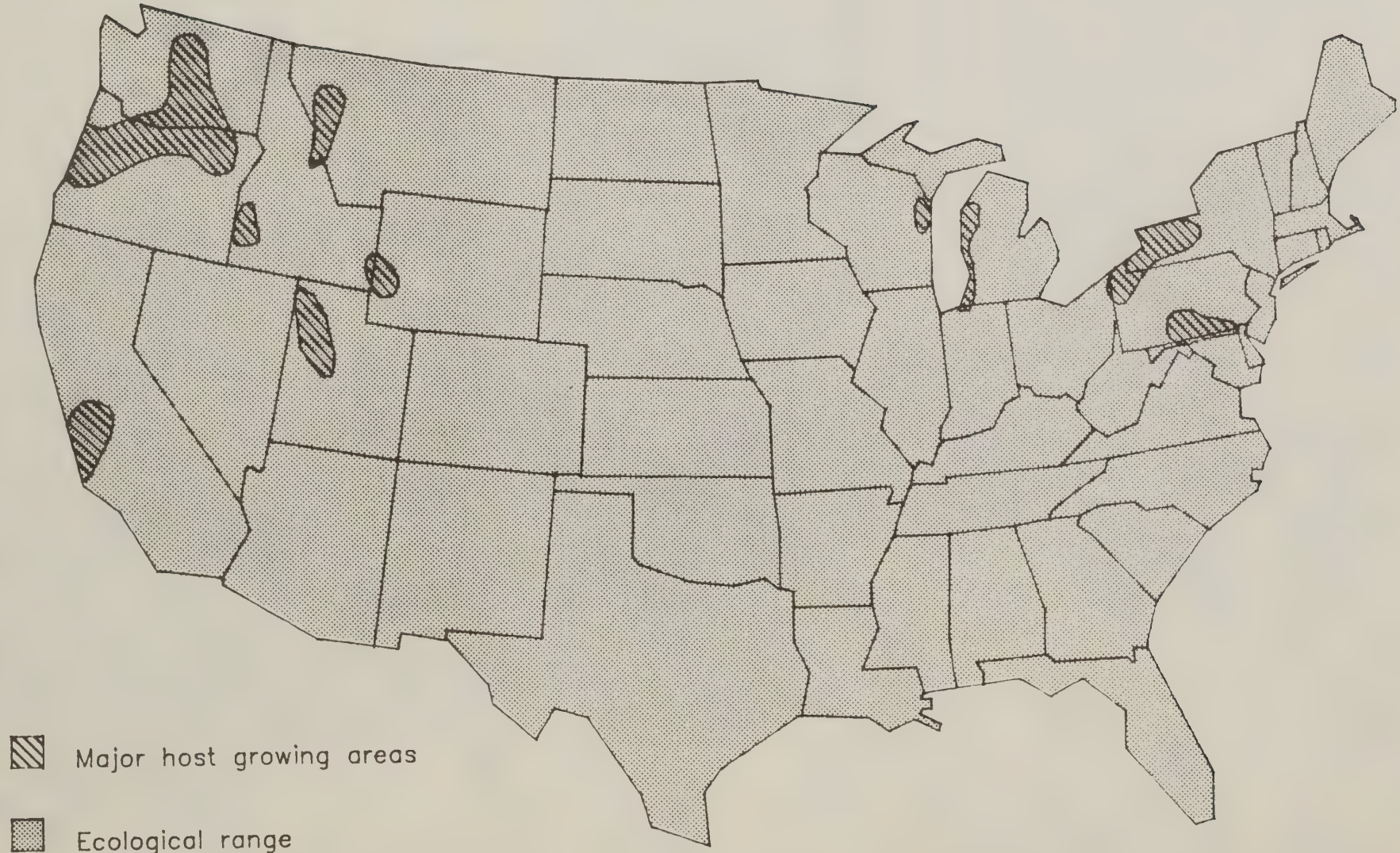
Rhagoletis cerasi

World distribution

Rhagoletis cerasi



Spodoptera littoralis Egyptian cotton leafworm

Hosts: Cotton, tobacco, alfalfa, soybeans, clover, vegetables

Distribution: See map

Biology: Spodoptera littoralis is a multivoltine species that does not enter a diapause stage, nor can it tolerate long periods of temperatures at 13°C or lower. S. littoralis can over-winter in southern Spain, but not in northern Italy or France.

The eggs are laid on the leaves of host plants and begin to hatch after 28.6 degree-days C (ddC) at a base temperature of 14.8°C. The optimal temperature for hatch is 28-30°C. Exposing the eggs to 13°C for eighteen days will result in complete egg mortality.

Newly-emerged larvae will feed on the leaves of cotton, but not on the large veins. Later instar larvae disperse widely, become nocturnal in habit, and will at times attack the young buds and cotton bolls. The larvae weaken the cotton plants and leave the plants susceptible to damage by the bollworms.

The optimal temperature for larval development is 25°C, and at a base temperature of 13°C, 257.1 ddC are required to complete the larval stage. Exposing the larvae constantly to 13°C does not prevent the larvae from forming prepupae, but all the prepupae will die.

Larvae pupate in the soil, and at the 13°C base temperature, male and female pupae complete their development in 177.1 and 153.5 ddC, respectively. Exposing the pupae to 13°C for seventy days will result in few adults emerging, and those that do emerge will be deformed and incapable of mating. Exposing the pupae to temperatures above 30°C will also result in poor survival. The females which do emerge will deposit many non-viable eggs. The optimal temperature for pupal survival is 20°C.

The adults emerge at night, with the males emerging about three hours after the females. The males can mate 5-6 times, but usually mate only once a night. The females will mate, at the most, twice. Few males will fly at temperatures below 13°C. The distance the adults migrate is unknown, although marked moths have been captured as far as 1500 meters from a release site. An infestation in France is thought to have come from migration of adults from overwintering areas in southern Spain.

Potential U.S. distribution: Where the average annual minimal temperatures are above 10°C (see map).

Recommended survey area: Major cotton producing states (see map). TX, CA, MS, AZ, AR, LA, OK, AL, TN, MO, NM, SC, GA, NC, FL.

Pheromone: 99.5:0.5 mixture of (Z,E)-9,11:(Z,E)-9,12-tetradecadienyl acetates
dispenser type - rubber septa
field life - 2 weeks, replace lure dispensers every 2 weeks.

Commercial source of pheromone dispensers: Zoecon Corporation, Pest-Select Int., Inc.

Traps: Zoecon Corporation (Pherocon-1-C); Pest-Select International, Inc. (Wing type)

Trap placement: Traps should be hung from stakes at approximately the height of the crop. As the season progresses, the trap should be raised as the crop height increases.

Recommended combinations: Egyptian cotton leafworm S. littoralis baits can be combined in traps with baits for the cotton leafworm (exotic) Spodoptera litura. Traps baited for S. littoralis and S. litura can be placed in any of the following crops: cotton, tobacco, soybeans, alfalfa, clover, or vegetable.

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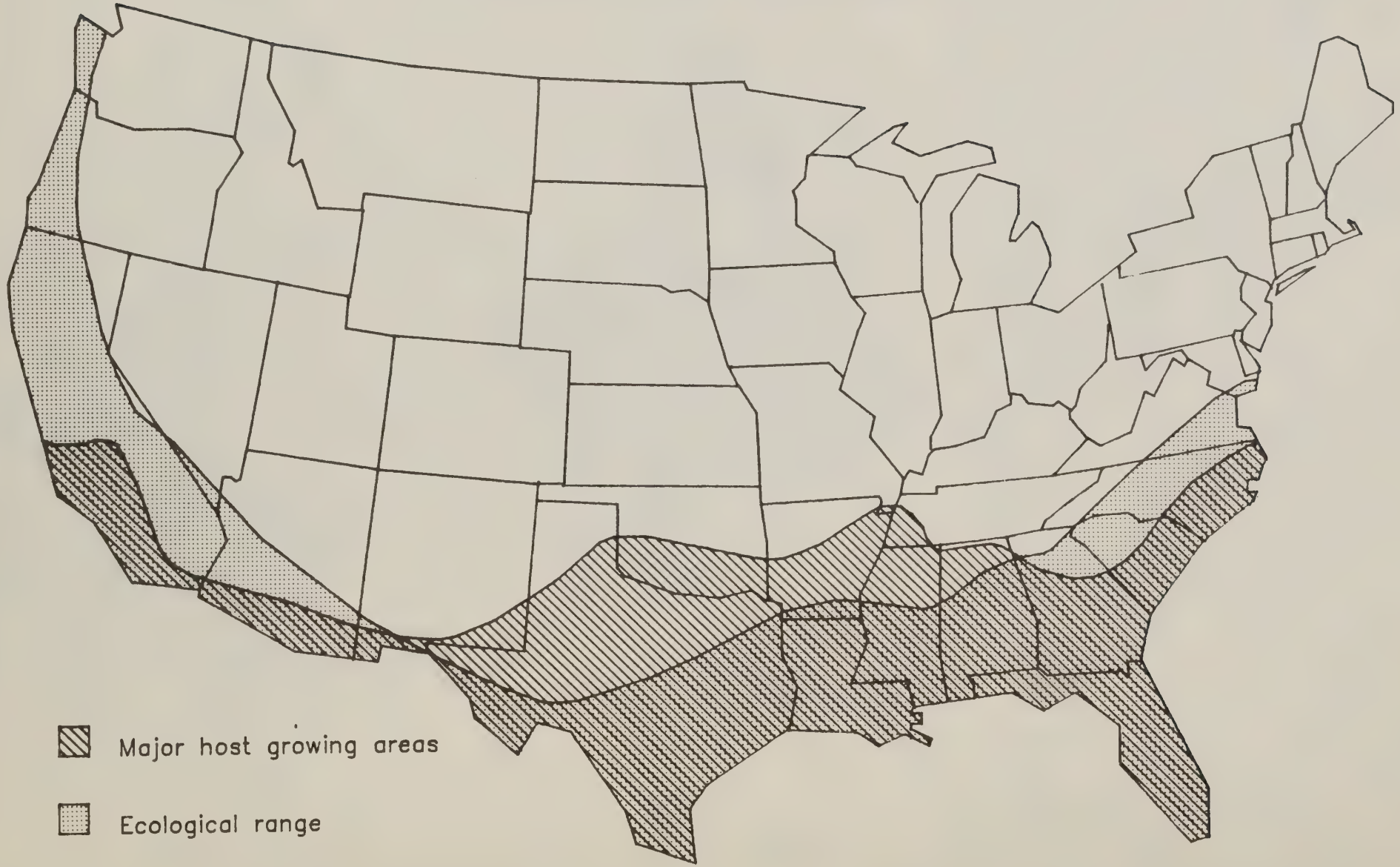
120 100 80 60 40 20 0 20 40 60 80 100 120 140 160 180
Spodoptera littoralis
World distribution

Spodoptera littoralis



Spodoptera litura African cotton leafworm (cluster caterpillar)

Hosts: Cotton, Tobacco, Grapes, Corn, Soybeans, Vegetables

Distribution: See map

Biology: Spodoptera litura is a multivoltine species with no known diapause stage. It has 2 generations/year in China, 4 to 5 generations/year in Japan and up to 8 generations/year in Taiwan. Temperatures of 10°C or lower will cause mortality in all the life stages with the most cold resistent stages capable of surviving -2°C for only 1 day.

A generation normally requires 526.3 degree-days C at a base temperature of 10.3°C. The eggs hatch in 4 days at 26.7°C. Newly hatched larvae are very susceptible to dry heat; consequently, they usually stay on the lower leaf surfaces during the day and feed at night. During the last two instars, the larvae feed only at night and find shelter during the day under the lowest leaves or in the soil at the base of the host plants. The larvae either defoliate the plant or cut it off like a cutworm.

At 28.6°C larvae pass through 6 instars in approximately 13 days and pupate within earthen cells. The pupal stage is completed in 7.3 and 6.1 days for male and female pupae, respectively, at 28.6°C.

The adults emerge at night between 11:00 p.m. and 3:00 a.m. The males can fly up to 5 km/night; however, flight is greatly reduced at temperatures below 20°C. The males will mate once each night and will avoid any females mated previously.

The females begin to deposit their eggs 2 to 3 days after emerging. The eggs are deposited at night in batches of up to 300 eggs on the under-surface of host leaves. A female can deposit from 6 to 9 batches of eggs over a 7 day life span.

Potential U.S. distribution: Where the average annual minimal temperatures are above 10°C (see map).

Recommended survey area: Major cotton producing States, and Florida (see map). TX, CA, MS, AZ, AR, LA, OK, AL, TN, MO, NM, SC, GA, NC, FL.

Pheromone: 88:12 mixture (Z,E)-9,11:(Z,E)-9,12 tetradecadienyl acetate
dispenser type - rubber septa
field life - 2 weeks, replace lure dispensers every 2 weeks

Commercial sources of pheromone dispensers: Zoecon Corporation; Pest-Select Int., Inc.

Traps: Zoecon Corp. (Pherocon-1-C); Pest-Select Int., Inc. (Wing-type)

Trap placement: Trap should be hung from stakes at approximately the height of the crop. As the season progresses, the trap should be raised as the crop height increases.

Recommended combinations: Cotton leafworm (S. litura) baits can be combined in traps with baits for the Egyptian cotton leafworm Spodoptera littoralis. Traps baited for S. litura and S. littoralis can be placed in any of the following crops: cotton, tobacco, soybeans, alfalfa, clover or vegetables.

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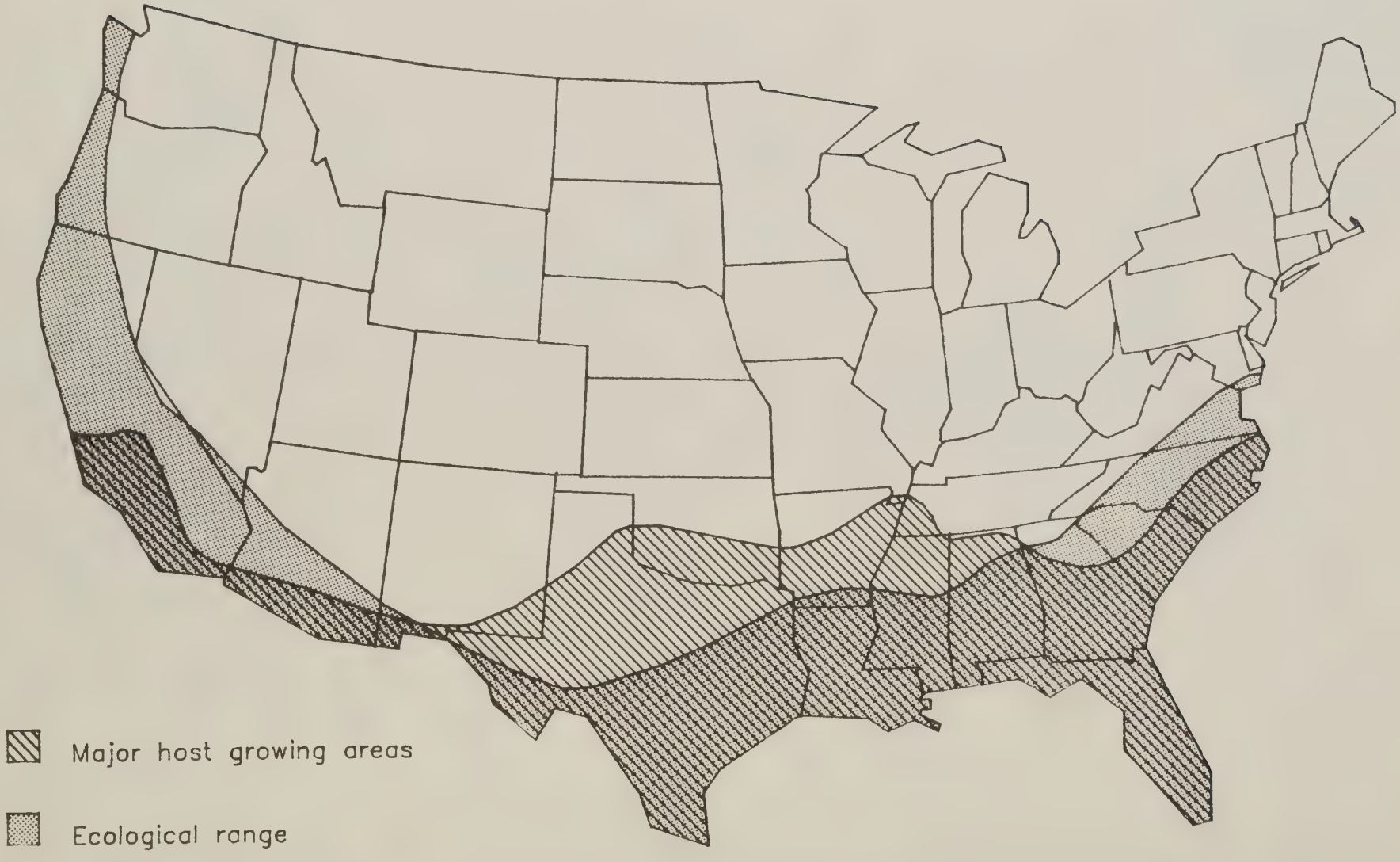
Spodoptera litura

World distribution

Spodoptera litura



Appendix 5a. Summary of the 1984 Detection Survey for Six Exotic Insect Pests

In 1984, a pilot scale detection survey using pheromone baited traps was conducted for 6 exotic insect species. The objectives of this pilot study were: 1) to determine what logistical problems were involved in conducting a national survey; 2) to identify potential problems with non-target organisms in traps; and 3) to conduct a survey (albeit at a low intensity) for the 6 exotic species.

In very brief summary, none of the 6 target exotic species was captured in any of the traps for which reports were completed. Logistics of the program were not smooth and the effort provided a learning opportunity for all personnel involved. Several shortcomings were clearly identified in a critique of the program and action has been taken to correct these.

The number of traps provided to each state for each species is presented on Table 1. A total of 2,337 traps for the 6 species were shipped either directly to state cooperators, or to area survey coordinators for further distribution. A total of 36 states cooperated in the program and all of these states surveyed for 2 or more species. Table 2 is a list of the states which have reported results; information was received on only 853 traps by March 15. This is partially due to the receipt of some trapping supplies in the field too late to be used. Obviously, one of the short falls of the 1984 program was reporting (or a mechanism for reporting). This summary is a compilation of information from those 855 trap reports that were submitted.

Tables 3 - 8 provide information on the number and frequency of non-target insects captured in traps baited for the six exotic species. This information is needed to predict which non-target species we might expect to encounter in future surveys so that taxonomic information for separating target species from similar-looking non-target species can be readied. This information is also of value in predicting in which areas trap "loading" by non-target species will occur. Remedial actions for problems may then be planned (i.e. more frequent trap checking); or a longer term solution may be sought by perhaps developing a more species-specific trap and/or bait. Construction of these tables involved considerable interpretation of data from the reports we received. Often, it was not possible to know if the number of non-targets reported were totals for the state or averages per trap and some judgments were made in order to utilize the information we received. Also, the scientific names of some specimens were unknown in the entomological literature available to us. Some spellings were reasonably close to known species, but we made no attempt to guess their true identity if the species was only rarely reported.

Results of the survey for Adoxophyes orana are presented in Table 3. The nontarget insect which was reported most often (18 reports) was Choristoneura rosaceana, the oblique-banded leafroller. Note that this non-target species was not reported from states east of Wisconsin. Interestingly, a second species, Pandemis limitata, which closely resembles C. rosaceana was reported by 3 states in the eastern portion of the country. The species that were captured second and third most frequently (seven reports each) were Lymantria dispar and Grapholita molesta. Gypsy moth was captured, not because of attraction by A. orana baits (actually A. orana pheromone inhibits gypsy moth capture), but most likely because of contamination of traps by L. dispar pheromone somewhere along the line. Although precautions were taken to prevent this type of cross-contamination, it apparently happened never-the-less and further precautions against contamination have been initiated. Grapholita molesta appears to be minimally attracted to A. orana baited traps. It can be easily separated from A. orana on gross morphological characteristics and, because of its small size, trap loading may only be significant where populations are dense. Two other species, Argyrotaenia velutinana and Pandemis pyrusana, were each reported twice. Argyrotaenia velutinana was reported from two states and may pose a problem for trap loading if it is locally very abundant. Pandemis pyrusana was reported only from Washington and it does not appear to pose a problem. A very small number of Grapholita prunivora and Orthodes comminis (not shown on Table 3) were also captured along with specimens of Coleoptera, Diptera, Hemiptera, Homoptera and Hymenoptera. Species which were identified only to Family (codes 9, 10, 11, 12) may pose a larger problem than anticipated in terms of trap-filling and identification. In 1985, surveys for A. orana should consider the species listed in Table 3 as non-targets that may be encountered.

The non-target species captured in traps baited for the plum fruit moth, Cydia funebrana, are listed in Table 4. Most frequently reported were the two species Grapholita prunivora and G. molesta. The composition of these two species' sex pheromones is very similar to that of C. funebrana; therefore, cross attraction is not surprising. Separation of these non-targets from C. funebrana by gross characteristics is also difficult, if not impossible, since wing scale patterns are destroyed when the specimen has been in a trap for any length of time. Genitalia taxonomic characteristics are available for separation of these species and work is currently being conducted by the French to develop a bait more specific for C. funebrana. The non-target species reported third most frequently was Pandemis limitata (4 reports). While the capture of non-target species seems to vary from state to state, large numbers of Tortricids can be expected in traps baited for C. funebrana.

Results of trapping for the false codling moth Cryptophlebia leucotreta are presented in Table 5. A Lepidopteran preliminarily identified in the genus Hyperstrotia, Family Noctuidae, Subfamily Acontiinae was captured the most frequently. Separation of it from the target species should not be difficult but trap "loading" will have to be considered, at least in the states where it was reported. Another Noctuid species tentatively placed in the genus Erastria, appears to present the same problem of trap loading but was only captured in one state, New Mexico. Species in the same family (Tortricidae) as C. leucotreta were only reported from Mississippi. Because the species were not identified, problems with separation of target and non-target species cannot be foreseen. Trap "loading" problems apparently will also have to be addressed.

Information on non-target species captured in traps baited for Lobesia botrana is limited (Table 6). Reports were only received for 63 traps and only two Lepidopteran non-target insects were recorded. From this small sample of data it appears that neither identification problems nor trap loading problems are of concern when surveying for this species.

Results of survey for the Egyptian cotton leafworm, Spodoptera littoralis, are presented on Table 7. The most commonly reported non-target insect was a Noctuid, which was possibly a member of the genus Erastria. It is interesting that these non-targets were also identified from traps placed for C. leucotreta and L. botrana which were deployed in the same states. Because of the different compounds used in formulating pheromone baits for these three target species, it is doubtful that specific attraction is involved in capturing this non-target insect. Perhaps this group of traps was contaminated with some chemical or simply this non-target was locally abundant and were blundering into traps. It was anticipated that we would capture some native Spodoptera spp. in S. littoralis traps. Although this occurred, the number of captures was relatively small and trap-loading should not present major problems; however, attention will have to be given to identification.

The only Dipteran species surveyed for was the European cherry fruit fly, Rhagoletis cerasi. The trap used for this species depends on visual cues and bait "host" odors for attraction. These cues are not species-specific and, as expected, several other Dipteran species were captured. Six of these were identified as species in the same genus as the target insect. For future surveys, the necessary taxonomic information for separating non-target from the target species will be developed. Trap loading, does not appear to be a problem.

Overall, the 1984 pilot scale survey provided information on where the methodology and logistics of the program needs to be improved. Prominent among these shortfalls is a lack of communication. It is important that cooperators be provided with necessary manuals, data sheets and a knowledge of what is expected of them to conduct a meaningful and orderly trapping program. Never-the-less adequate experience and insight into the use of these attractants has been gained through this pilot survey to expand the effort. Accordingly, we recommend that operational surveys be conducted for these species over the next few years to ensure that any incipient, yet unrecognized populations that may exist are detected. When that is completed, future surveys for these species only need to be done intermittently.

Table 1. Number of pheromone traps provided for use in 1984 pilot scale exotic pest survey.

STATE CODE	Adoxophyes orana ADOX	Spodoptera littoralis ECL	Cryptophlebia leucotreta FCM	Lobesia botrana LB	Cydia funebrana PFM	Rhagoletis cerasi RC
AL		10	10			
AR		5	5	5		
AZ	25	25	25	25	25	10
CA	50	50	50	75	80	25
CT	5			5	5	
DE	15				15	
FL		62	62			
GA		20	50			
OR	30			20	30	32
ID				5	5	6
WA	45	25				50
MT					10	8
WI	15			15	15	15
MI	20			20	20	20
OH	10			10	10	20
PA	5			2	5	4
OK		12	12			
NM		18	18	18		18
TX		18	18			
ME	5				5	
MA	5				5	
NH	5				5	
NY	14			14	14	
RI	5				5	
VT	5				5	5
NC	20	66	66	30	20	
IN	10				10	
WV	15				15	
NJ	15				15	
IL	8				8	2
UT	15				15	15
VA	60			10	60	
MD	100				100	
LA		6	6			
MO	2	1		5	2	
MS		100	100			
Totals	504	418	422	259	504	230

Table 2. Number of pheromone traps placed in the 1984 pilot scale exotic pest survey program from which data were obtained.

STATE CODE	Adoxophyes orana ADOX	Spodoptera littoralis ECL	Cryptophlebia leucotreta FCM	Lobesia botrana LB	Cydia funebrana PFM	Rhagoletis cerasi RC
AL		9	9			
AR			4			
AZ						
CA						
CT	3				2	
DE	5				5	
FL						
GA						
OR	30				30	24
ID	5			5	5	6
WA	48	15		20		50
MT					8	8
WI	12			12	12	12
MI						
OH	9			7	8	11
PA	5			2	5	4
OK			12			
NM		12	18	7		18
TX		17	7			
ME	5				5	
MA						
NH	5				5	
NY						
RI	5				3	
VT						
NC						
IN						
WV	15				15	
NJ	13				15	
IL						
UT						
VA	17			10	18	
MD	22				25	
LA			4			
MO					2	
MS		91	97			
Totals	199	144	151	63	163	133

Table 3. Total number of non-target insects captured in traps baited for summer fruit tortrix, Adoxophyes orana. Columns (1-8) arranged in descending order of frequency of positive trap reports.

State	No. of traps	Species Codes 1	2	3	4	5	6	7	8	9	10	11	12
CT	3			13		9			4				
DE	5		4					80					
ID	5	48											
MD	22			11	3								
ME	5		20	68									
NH	5												
NJ	13									1	37	56	17
OH	9												
OR	30	2											
PA	5												
RI	5		171			20							
VA	17									P[1]/			
WA	48	72					13			3			
WI	12	96											
WV	15												
Totals	199	218	195	92	3	29	13	80	4	4+	37	56	17
No. of positive reports		18	7	7	3	2	2	1	1				

1/ Positive insect capture. Numbers not reported.

Insect Codes:
1 - Lepidoptera:Tortricidae Choristoneura rosaceana
2 - Lepidoptera:Lymantriidae Lymantria dispar
3 - Lepidoptera:Tortricidae Pandemis limitata
4 - Lepidoptera:Tortricidae Grapholita molesta
5 - Lepidoptera:Tortricidae Argyrotaenia velutinana
6 - Lepidoptera:Tortricidae Pandemis pyrusana
7 - Lepidoptera:Gracillariidae Phyllonorycter blancardella
8 - Lepidoptera:Tortricidae Sparganothis dilulicostana
9 - Lepidoptera:Noctuidae
10 - Lepidoptera:Olethreutidae
11 - Lepidoptera:Pyralidae
12 - Lepidoptera:Tortricidae

Table 4. Total numbers of non-target insects captured in traps baited for Cydia funebrana. Columns 1-9 arrayed in descending order of frequency of positive trap reports.

State	No. of traps	Number of specimens of each species captured													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
CT	2	97	269	10											
DE	5				222										
ID	5								12						
MD	25	274	203	39						6					
ME	5	446													
MO	2														
MT	8														
NH	5														P[1]/
NJ	15										1	2	500	23	1
OH	8														
OR	30														
PA	5						P[1]/								
RI	3					162	49	19							
VA	18										P[1]/			P[1]/	P[1]/
WI	12														
WV	15														
Totals	163	817	472	49	222	162	49+	19	12	6	1+	2	500	23+	1+
No. of positive reports		16	11	4	1	1	1	1	1						

1/ Positive insect capture. Numbers not reported.

Lepidopteran species reported of which 2 or less were captured in a single trap: Caenurgina erechtea, Plathypena scabra, Prodenia praefica, Syngrapha falcifera.

Other orders of insects which were reported: Coleoptera, Diptera, Homoptera, Hymenoptera.

Insect Codes:

1 - Lepidoptera:Tortricidae Grapholita prunivora
2 - Lepidoptera:Tortricidae G. molesta
3 - Lepidoptera:Tortricidae Pandemis limitata
4 - Lepidoptera:Gracillariidae Phyllonorycter blancardella
5 - Lepidoptera:Tortricidae Archips rosaceana
6 - Lepidoptera:Tortricidae Argyrotaenia velutinana
7 - Lepidoptera:Lymantriidae Lymantria dispar
8 - Lepidoptera:Noctuidae Spodoptera ornithogalli
9 - Lepidoptera:Tortricidae Laspeyresia pomonella
10 - Lepidoptera:Geometridae
11 - Lepidoptera:Noctuidae
12 - Lepidoptera:Olethreutidae
13 - Lepidoptera:Pyralidae
14 - Lepidoptera:Tortricidae

Table 5. Total number of non-target insects captured in traps baited for *Cryptophlebia leucotreta*.

State	No. of traps	1	2	3	4	5
AL	9					
AR	4	45				
LA	4	41+				
MS	97			507	16	P[1]/
NM	18	33	24			
OK	12	120+				
TX	7	5+				
Totals	151	244+	24	507	16	P+
No. of positive reports		18	3	15	4	1

1/ Positive insect capture. Numbers not reported.

Insect Codes: 1 - Lepidoptera:Noctuidae *Acontiinae Hyperstrotia spp.*
2 - Lepidoptera:Noctuidae *Acontiinae Erastria spp.*
3 - Lepidoptera:Tortricidae
4 - Lepidoptera:Tortricidae *Phaneta spp.*
5 - Tortricidae:Pyralidae *Crambus spp.*

Table 6. Total number of non-target insects captured in traps baited for the grapevine moth, *Lobesia botrana*.

State	No. of traps	1	2	3
ID	5			
NM	7	4	48	
OH	7			
PA	2			P[1]/
WA	20			
WI	12			
VA	10			
Totals	63	4	48	P
No. of positive reports		2	4	1

1/ Number of specimens captured not reported.

Insect Codes: 1 - Lepidoptera:Noctuidae *Acontiinae* or *Erastria spp.*
2 - Lepidoptera:Geometridae
3 - Diptera:Callphoridae

Table 7. Total numbers of non-target insects captured in traps baited for Spodoptera littoralis. Columns (1 - 5) arrayed in decreasing order of frequency of trap reports.

State	No. of traps	1	2	3	4	5
AL	9					
MS	91		2	2	1	
MN	12	100				
TX	19	82				
WA	15					15
Totals	146	182	2	2	1	15
No. of positive reports		7	2	2	1	1

Insect Codes:
1 - Lepidoptera:Noctuidae Acontiidae or Erastria spp.
2 - Lepidoptera:Noctuidae Spodoptera spp.
3 - Lepidoptera:Noctuidae Spodoptera ornithogalli
4 - Lepidoptera:Noctuidae Spodoptera frugiperda
5 - Lepidoptera:Pyralidae Udea profundalis

Table 8. Total numbers of non-target insects captured in traps baited for Rhagoletis cerasi. Columns 1-6 arrayed in descending order of frequency of positive trap reports.

State	No. of traps	1	2	3	4	5	6	7	8
ID	6								
MT	8		6	9					
NM	18	39					7	36[1/]	
OH	11								
OR	24	1	4						
PA	4							P[2/]	P[2/]
WA	50		1	3	1	1			
WI	12				4				
Totals	133	40	11	12	5	1	7	36+	0+
No. of positive reports		6	4	3	2	1	1		

1/ Condylostylus philicornis identified from several traps. All reports of this species were from New Mexico.

2/ Positive insect capture. Numbers not reported.

Insect Codes:
1 - Diptera:Tephritidae Rhagoletis completa
2 - Diptera:Tephritidae R. zephyria
3 - Diptera:Tephritidae R. indifferens
4 - Diptera:Tephritidae R. pomonella
5 - Diptera:Tephritidae R. basiola
6 - Diptera:Tephritidae R. juglandis
7 - Diptera
8 - Homoptera

Project Number: AW 1.1.1
Project Title: Evaluation of the Alfalfa Weevil Parasite Redistribution Program
Report Period: October 1, 1983 to September 30, 1984
Report Type: Interim
Project Leader: Philip C. Kingsley

Introduction:

This project was initiated in 1981 to evaluate the effects of AW parasitoid establishment on alfalfa weevil populations and subsequent changes in the local economics of growing alfalfa. The movement of principally two species, Bathyplectes anurus and Microctonus aethiopoides, from eastern areas, where these species have been well established, to areas further west has been coordinated from the APHIS Biocontrol Laboratory in Niles, Michigan. Recovery surveys, three years post-release, indicate wide-spread success of the distribution methods.

The movement of these two species has also been documented in our intensive sampling of five areas, each with six sites and five fields per site (Figure 1). Areas I - IV have been sampled for four years whereas the Nebraska area was added to the survey in 1983. In addition, we are beginning to unravel some possible relationships between the various parasitoids and their host.

Methods:

Survey methods, as described in the 1984 Evaluation Handbook, remained the same as the previous year. For the first time, however, data were collected on the incidence of disease in AW larvae and adults as they were being dissected for parasitoid species determination and parasitism rates at the Niles laboratory.

Lancaster county (site C) Pennsylvania, was not surveyed this year due to an avian influenza quarantine.

Results:

Large populations of weevils were recorded in some western sites; ten fields had peak larval densities exceeding 1000 per 100 sweeps. In a Nebraska site F field, for example, larvae numbered 3300 per 100 sweeps. In contrast, fields in Pennsylvania and New Jersey had few weevils with an average peak larval density of only 52 per 100 sweeps (Table 1).

The following county records were recovered from the Evaluation survey:

IL,D	Mason Co.	Microctonus aethiopoides
IL,E	De Witt Co.	Bathyplectes anurus
NE,C	Polk Co.	M. aethiopoides
NE,E	Adams Co.	B. anurus

Personnel at the Niles laboratory dissected 9664 and 23368 AW adults and larvae, respectively, from the Evaluation Survey. In addition, 8737 Bathyplectes coccoons were reared at the three cooperating laboratories.

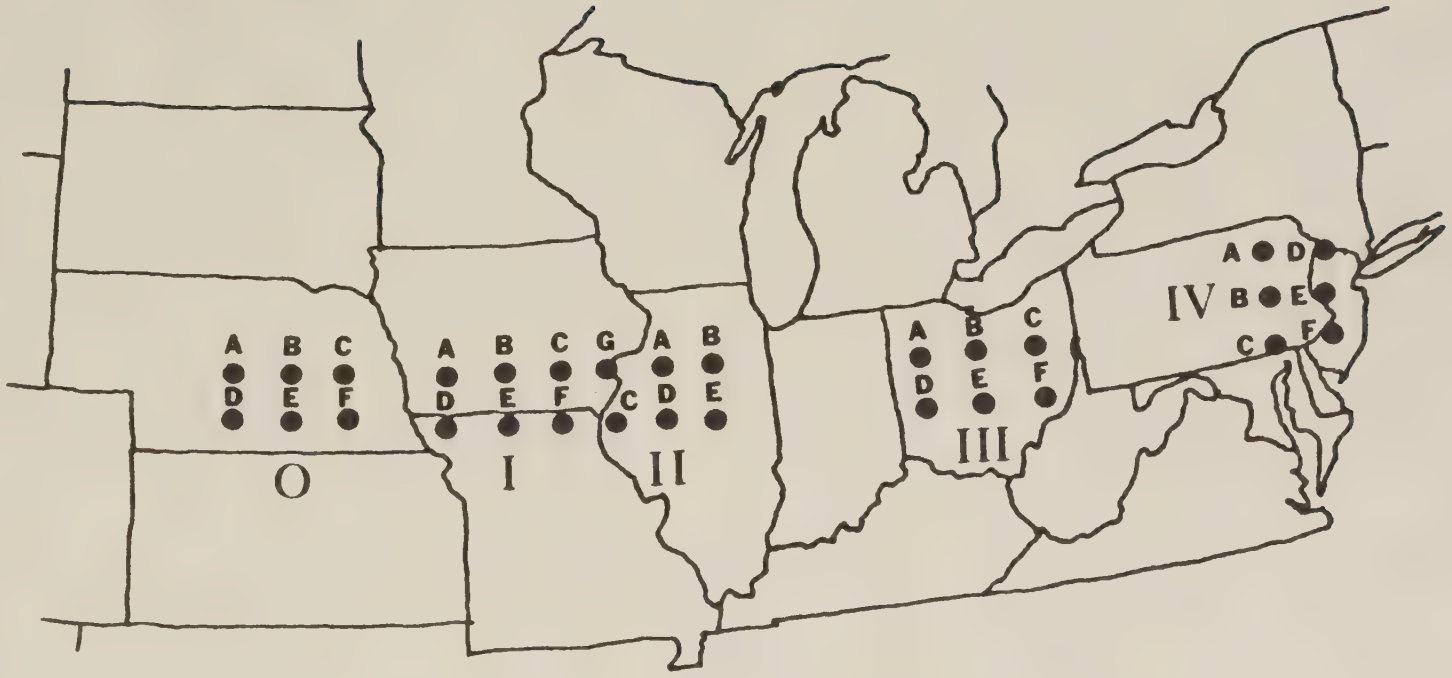


Figure 1. 1985 AW Evaluation Areas and Sites (4 Areas, 6 Sites/Area, 5 Fields/Site).

Table 1. Four-year area summary for peak larval densities of the alfalfa weevil, parasite species diversity, and percent parasitism.

Area	Mean (SE) Peak Larval Density (#/100 Sweeps)				Mean (SE) Number of Parasite Species/Site			
	1981	1982	1983	1984	1981	1982	1983	1984
O NB			262.3ab (26.5)	706.7a (164.93)			1.5a (0.22)	1.5a (0.22)
I MO, IA	619.5b[1] (105.0)	193.3ab (36.2)	351.7b (56.1)	253.0b (28.56)	1.7a (0.21)	2.3a (0.21)	2.3a (0.33)	2.0a (0.00)
II IA, IL	846.3b (199.3)	251.5ab (59.0)	207.0a (33.8)	345.4b (77.43)	2.2a (0.17)	2.5a (0.33)	2.5a (0.34)	3.0b (0.45)
III OH	92.1a (22.3)	97.5a (22.9)	161.8a (8.4)	245.8b (31.12)	3.5b (0.22)	3.8b (0.31)	3.5b (0.34)	3.3b (0.24)
IV NJ, PA	218.1a (39.9)	381.7b (87.9)	171.1a (34.8)	52.8b (13.67)	4.5c (0.29)	4.0b (0.26)	3.8b (0.17)	3.0b (0.00)

Area	Adult Parasitism (Percentage)				Larval Parasitism (Percentage)			
	1981	1982	1983	1984	1981	1982	1983	1984
O NB			0.04c	0.07a			24.8c	22.2b
I MO, IA	2.3a	13.2a	16.5a	22.7d	14.4c	18.1a	13.8a	15.2a
II IA, IL	39.1b	35.9c	35.1b	17.2bc	8.1a	40.0c	29.0d	25.5c
III OH	32.0b	26.6b	18.3a	20.2dc	11.6b	26.0b	15.7b	22.6b
IV NJ, PA	26.2b	32.6bc	19.1a	14.0b	30.9d	26.3b	27.1cd	14.1a

1. Values in columns followed by the same letter are not significantly different at the 95 percent level of confidence. Peak larval densities and the number of parasite species were tested with a Newman-Keuls multiple comparison test, while parasitism frequencies were tested using a Chi-square analysis.

During the four years of our survey, each area has shown a unique and shifting parasitoid species complex. All five species we survey for, Microctonus aethiopoides (MA), M. colesi (MC), Bathyplectes anurus (BA), B. curculionis (BC), and Tetrasticus incertus (TI), occurred in area IV (Pennsylvania, New Jersey) with some regularity. During the 1981 survey for example, there was an average of 4.5 species per site (Table 1). Of note, in area IV are the relative numbers of BA and BC. Established in this area since the early 1960's, BC has been replaced (in terms of importance) by BA, which was released approximately five years later. The recovery rate for BC is still fairly high in the area IV fields (Figure 6) but this species accounts for only a very small percentage of the total larval parasitism [less than 6% (27/477) in 1984, Figure 7].

Bathyplectes curculionis dispersed very rapidly after its release in the east and has been established in all five areas since the early 1970's. Where BC occurs without its congener BA (areas 0, I, II), its parasitism rates are much higher than those found in area IV. In Ohio (area III), BA is increasing its range rapidly through a combination of natural dispersal and project distributions. This parasitoid now occurs in 63.3% of the Ohio evaluation fields compared with 37.9% in 1981 (Figure 5). The relative abundance of the two species in the six Ohio sites is indicated in Figure 8. It appears that as BA moves west across the state, it will also become the dominant larval parasitoid as it has in area IV.

Bathyplectes anurus is also being recovered in the three western areas, but as yet, is still in the very early stages of establishment. In Nebraska, for example, a state record and nine county records have been recovered within the last two years through the Evaluation Survey and the Niles Parasitoid Establishment Survey. These establishments are undoubtedly due to the redistribution program.

Of the two adult parasitoids, MA seems to be the most important species in our survey areas. Microctonus colesi occurs sporadicaly in some fields but never in large numbers. In 1982, for instance, MC was recovered in 72% of the Ohio fields (Figure 5) but accounted for only 6% (17/287) of the total adult parasitism. In contrast, where MA has become well established (areas II, III, IV; Figures 4-6) it maintains high parasitism rates (Figure 7).

This build up of MA is being documented in area I (MO, IA). This species has become firmly established there during our four survey years, with an increase in field recovery from 36% of the 30 survey fields in 1981 to 100% in 1984 (Figure 3). Similarly, parasitism rates have also steadily increased from 6% to 25% over the four years (Figure 7). Since BC has remained at a fairly constant parasitism rate and BA is not yet a factor, this area allows for some unique comparisons between parasitoid and host populations.

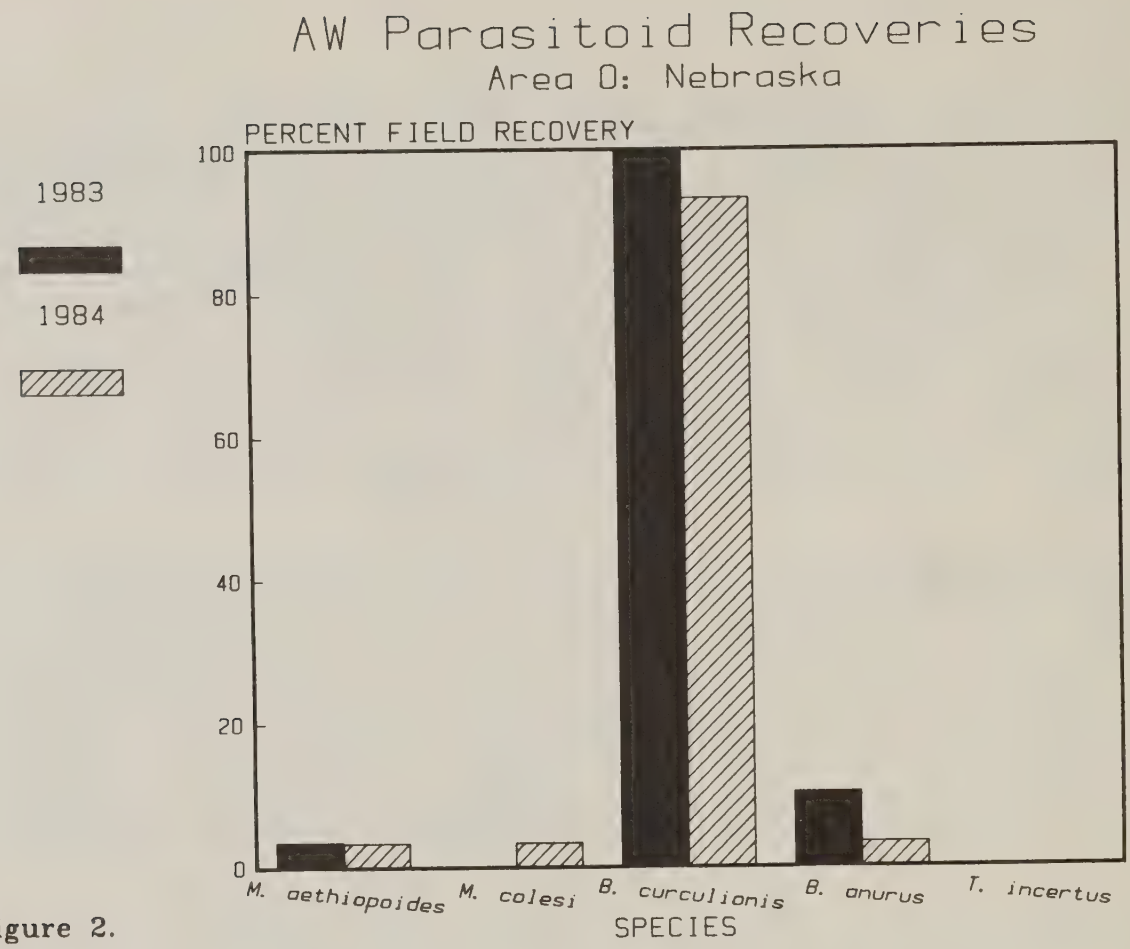


Figure 2.

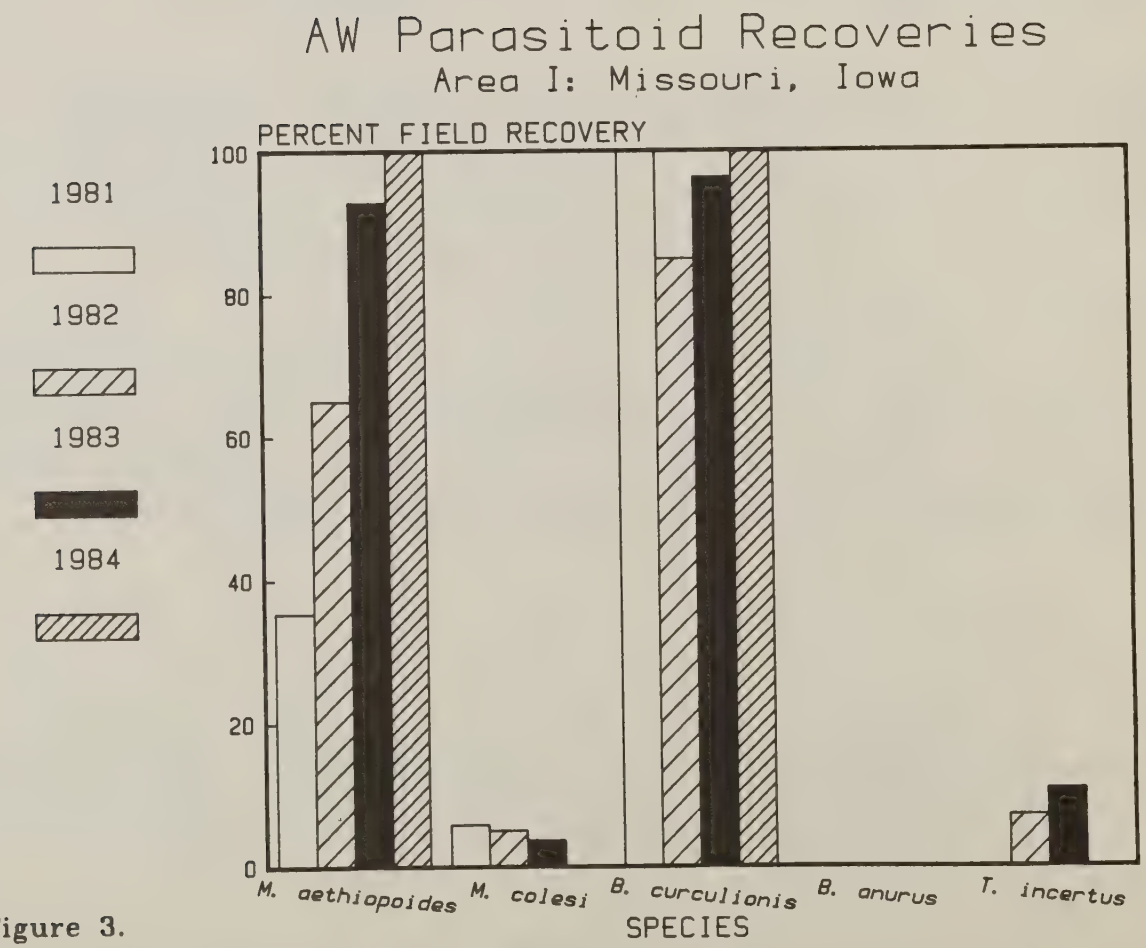


Figure 3.

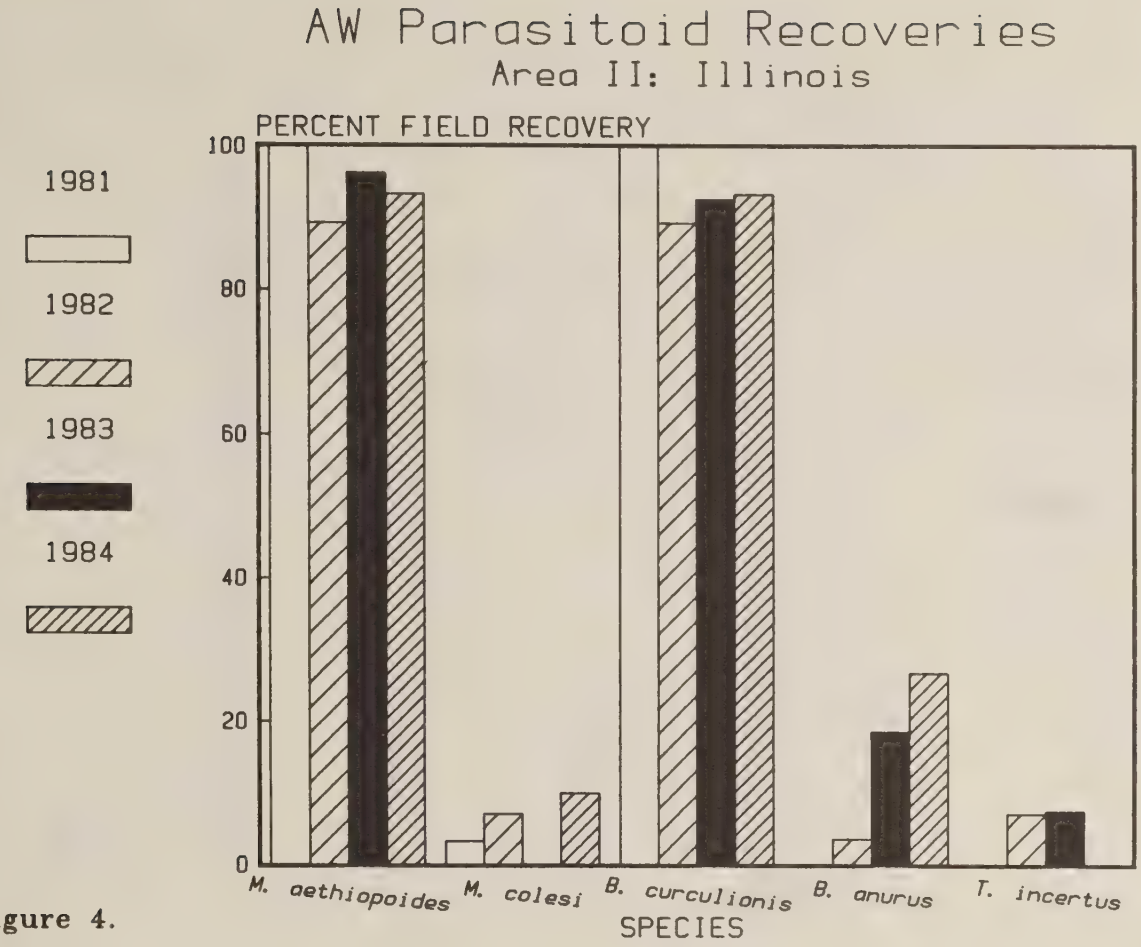


Figure 4.

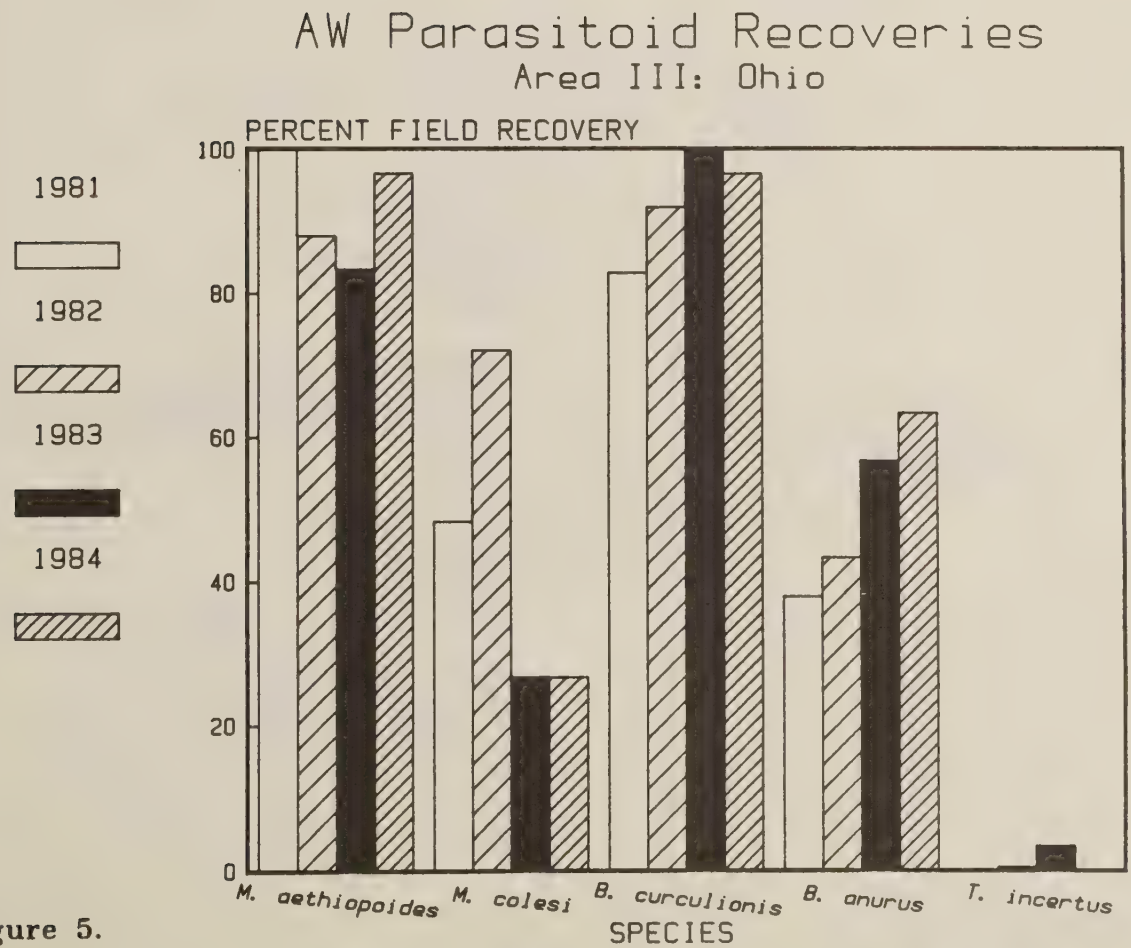


Figure 5.

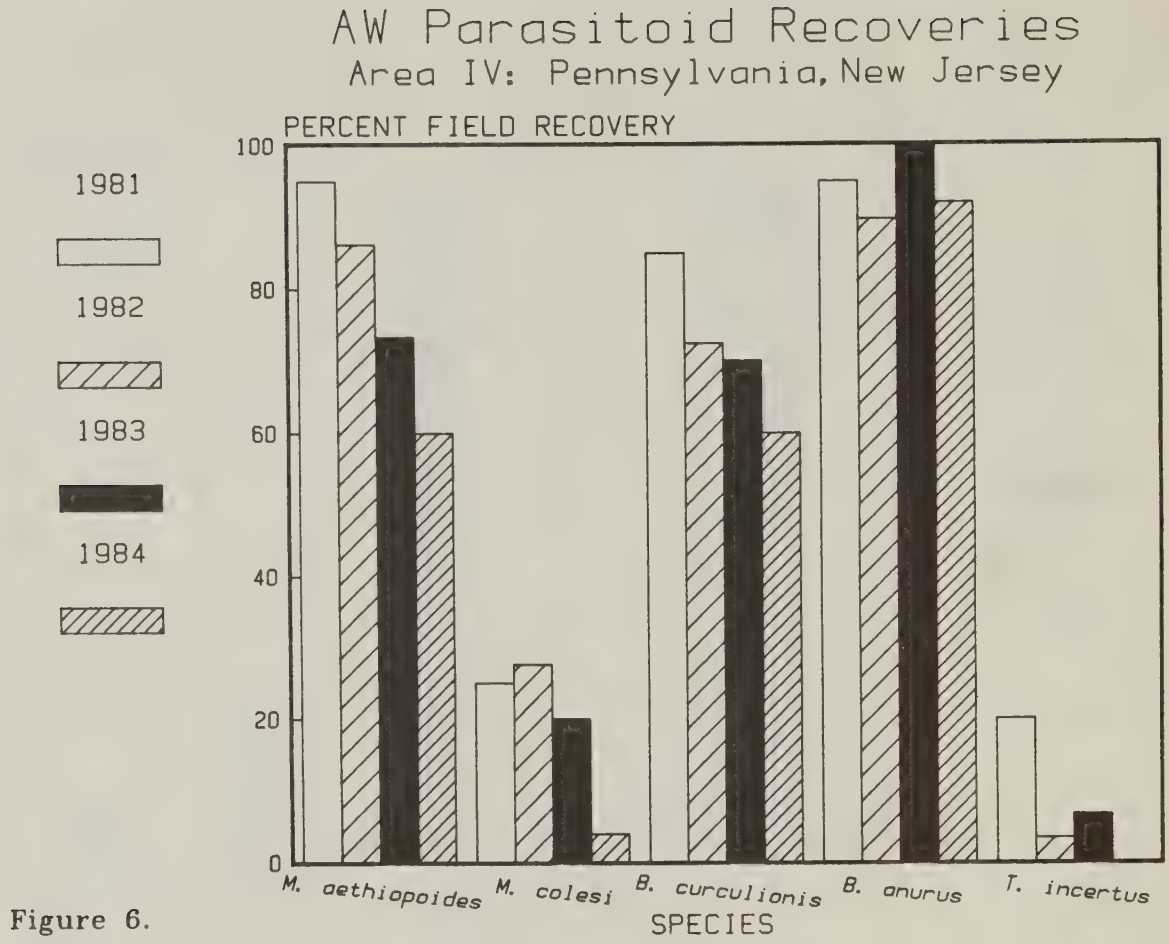


Figure 6.

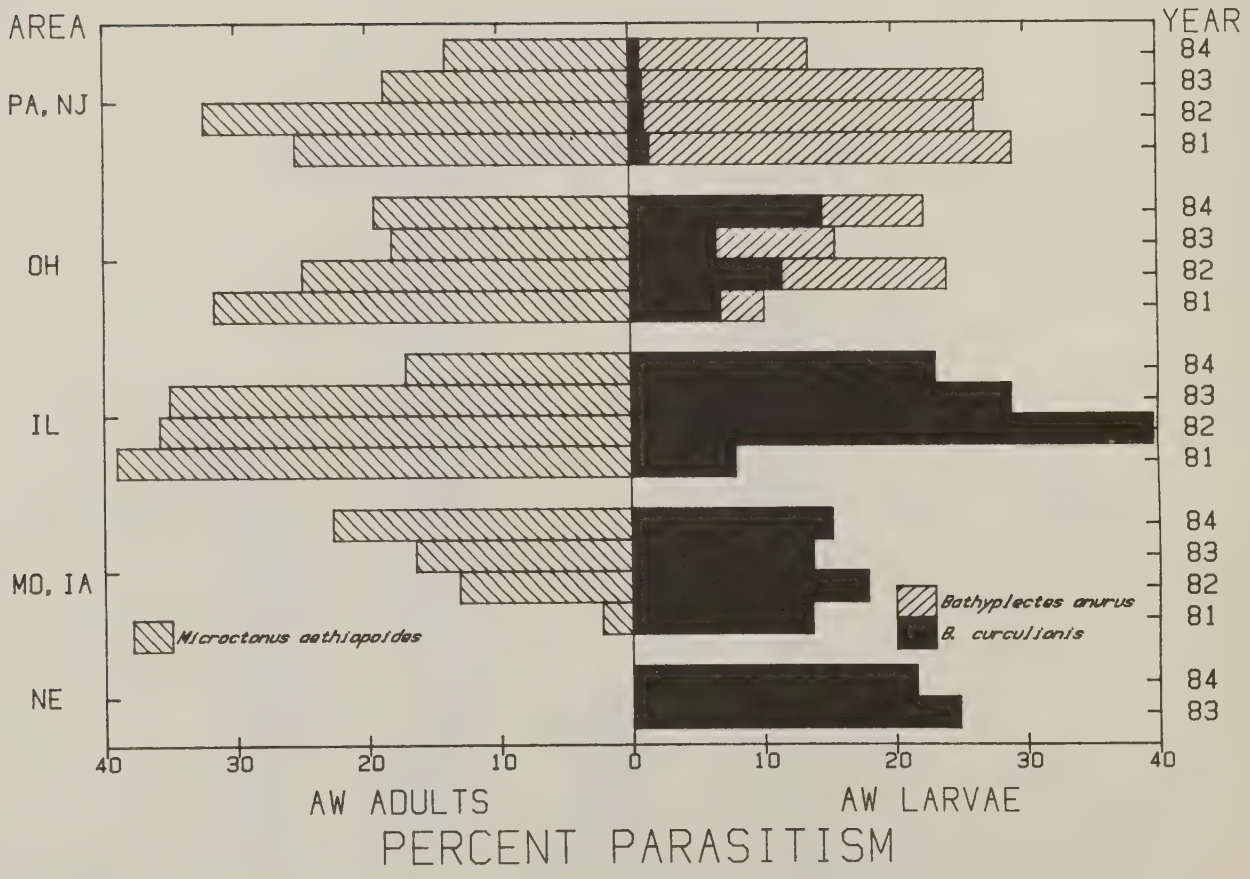


Figure 7.

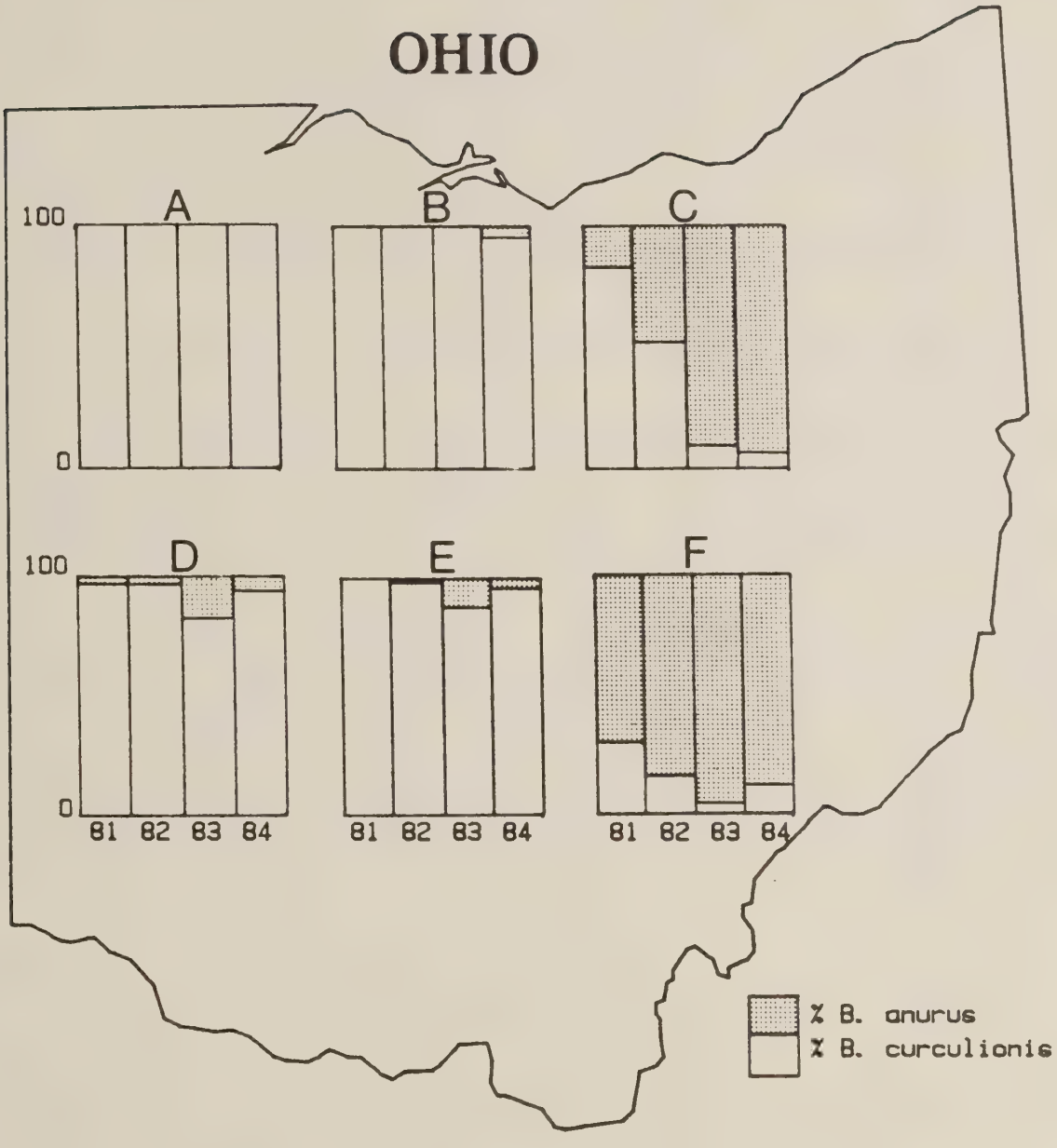


Figure 8. Relative frequencies of the two AW larval parasitoids Bathyplectes anurus and B. curculionis reared from six evaluation sites in Ohio, by year. The total number reared in 1981, 1982, 1983 and 1984 was 1495, 2432, 2426 and 2478 respectively.

If we compare the mean peak densities of adult and larval weevils in those MO, IA fields[1] where MA was recovered vs not recovered, densities are significantly lower where MA was found (T-test, $P \leq .05$).

	Mean Peak Densities (SE) #/100 sweeps		
	Larvae	Adults	No. of Fields
MA recovered	319.0 (32.63)	16.5 (2.31)	88
MA not recovered	509.6 (87.45)	26.2 (4.22)	32

1 Each year a field was considered separately, i.e. 5 fields/6 sites/4 years = 120 fields.

In terms of percent parasitism, or the relative abundance of MA in a field, the mean peak AW densities were compared for those fields with different levels of parasitism i.e., 0-10%, 10-20%, etc. There appears to be a strong relationship between the percentage of adult parasitism by MA in a field and peak weevil populations (Figure 9).

As mentioned above, the incidence of disease (Erynia sp.) was recorded as weevil larvae and adults were being dissected for parasitism determination. Table 2 summarizes the relative abundance of the disease on a field basis, that is, the percentage of area fields in which the disease was recovered from either larvae or adults. Also indicated is the overall percentage of weevils parasitized. As seen below, when the mean peak larval densities of fields where disease was recovered was compared with those fields where disease was not detected, the fields with diseased weevils had significantly higher densities (T-test, P .05).

	Mean Peak Densities (SE) #/100 sweeps		
	Larvae	Adults	No. of Fields
Fungus recovered	373.3 (51.50)	6.6 (0.47)	116
Fungus not recovered	148.8 (29.06)	3.3 (0.64)	29

There was also a slight ($R^2 = .28$), although significant ($P \leq .000$), positive correlation between the percentage of adults diseased and peak larval densities in each field. This density dependence is to be expected as higher populations may lead increased infection rates for a number of reasons (stress, transmission). We will be watching for a negative correlation between this years disease levels and next years AW densities.

A four year phenological record of AW populations and parasitism is given for each area in Figures 10 - 14. In addition, detailed information on AW densities and parasitism rates by species summarized on a site and area basis can be found in four annual archive data reports.

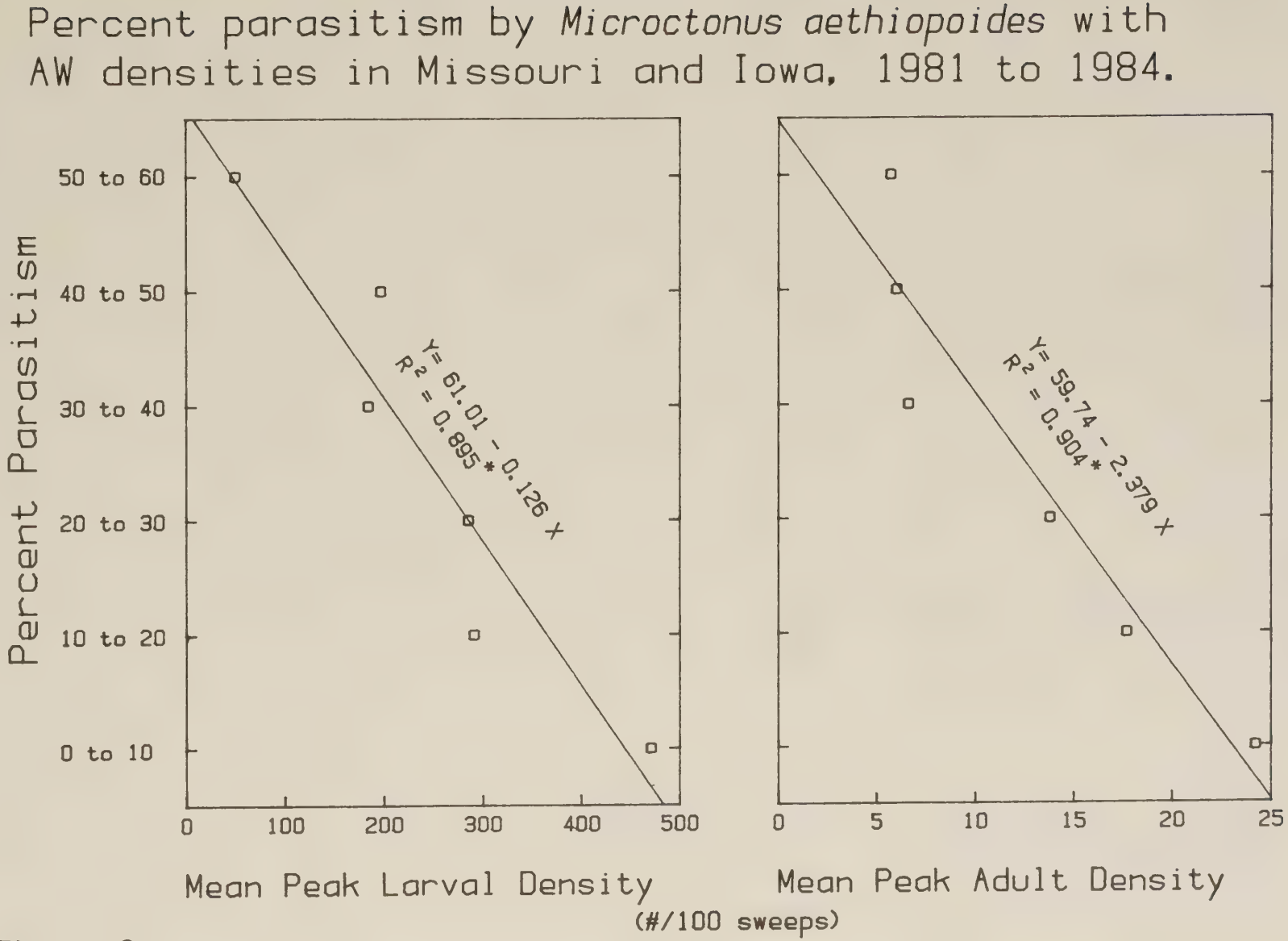

Percent parasitism by *Microctonus aethiopoides* with AW densities in Missouri and Iowa, 1981 to 1984.
Percent Parasitism
50 to 60
40 to 50
30 to 40
20 to 30
10 to 20
0 to 10
Y= 61.01 - 0.126 X
R² = 0.895 *
0
100
200
300
400
500
Mean Peak Larval Density
Y= 59.74 - 2.379 X
R² = 0.904 *
0
5
10
15
20
25
Mean Peak Adult Density
(#/100 sweeps)

Figure 9.

Table 2. Field recovery and infection rates of diseased alfalfa weevil adults and larvae during the 1984 AW Evaluation Survey.[1/]

Area	% of fields[2/] with diseased weevils	% of larvae diseased	% of adults diseased
NE	90.0	9.0	4.4
MO, IA	93.3	10.2	1.9
IL	86.7	7.0	1.3
OH	63.3	3.0	0.4
PA, NJ	64.0	8.9	3.4

1/ Determined as weevils were being dissected for parasitism data.

2/ 30 fields per area, except PA, NJ with 25.

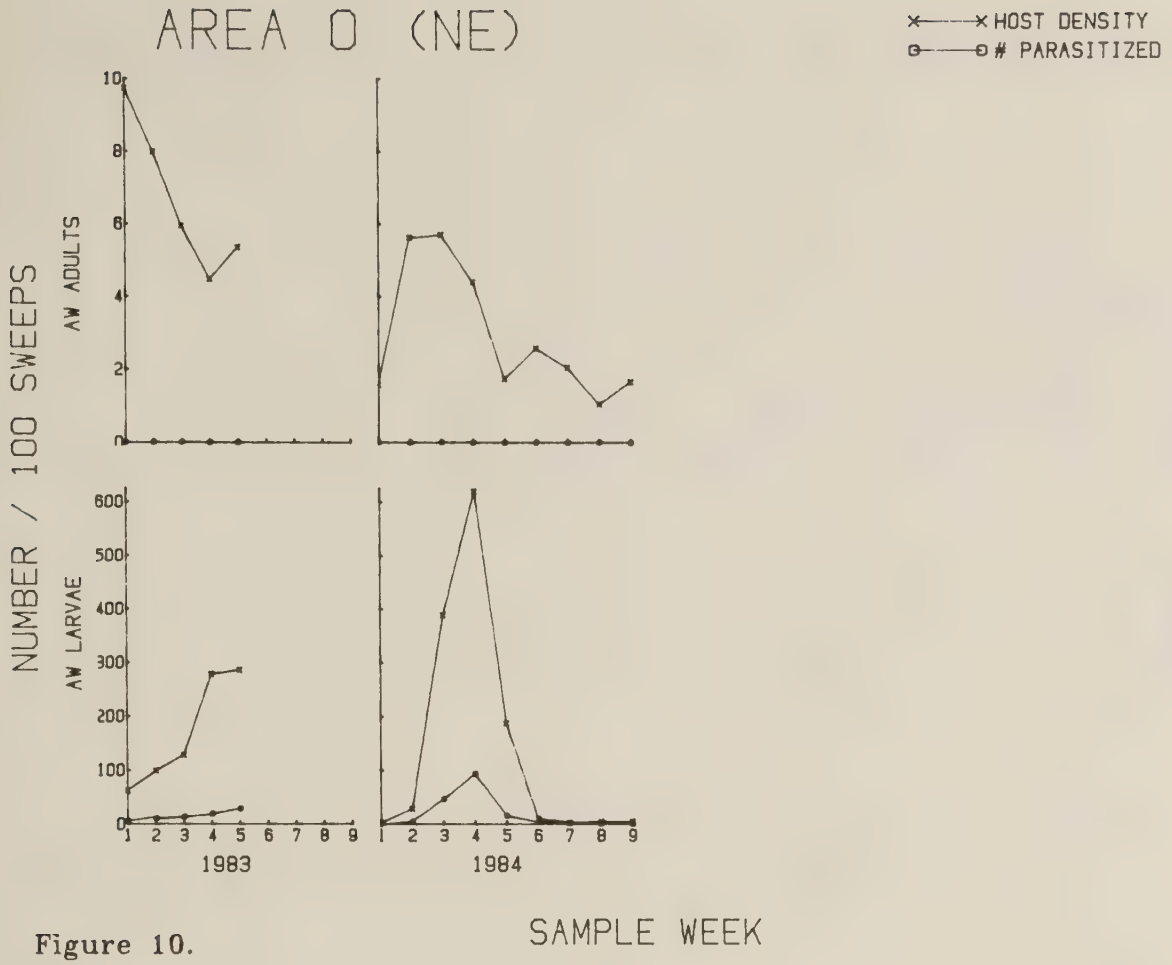


Figure 10.

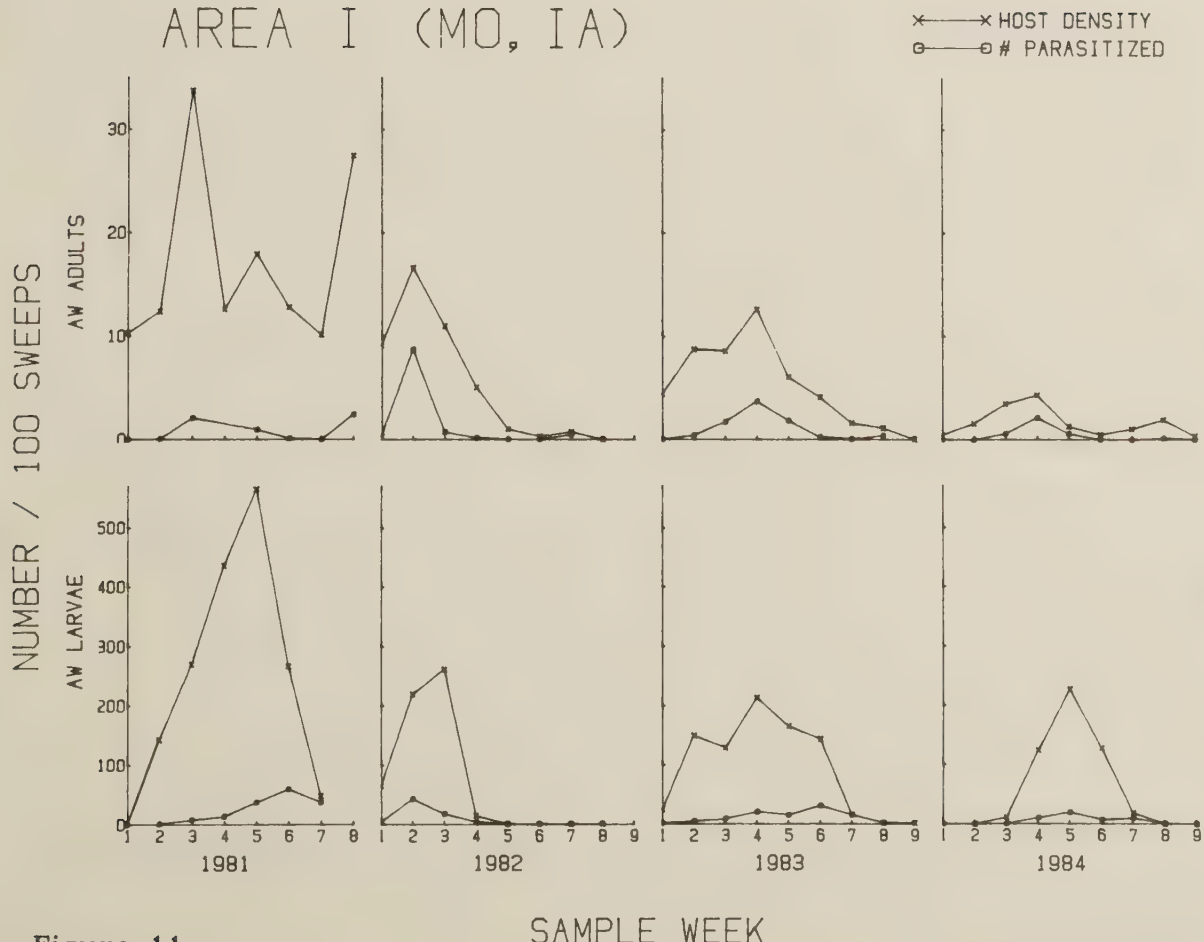


Figure 11.

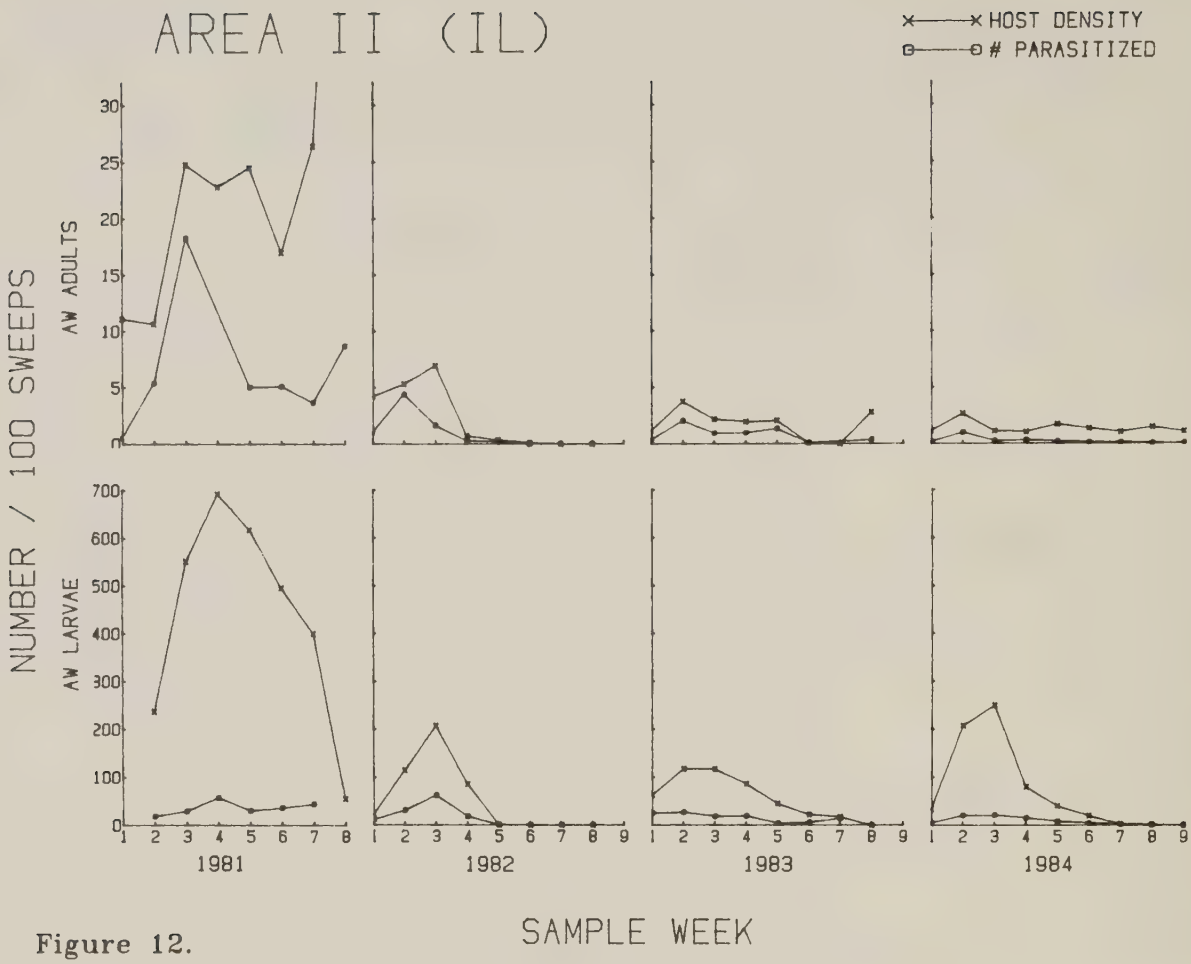


Figure 12.

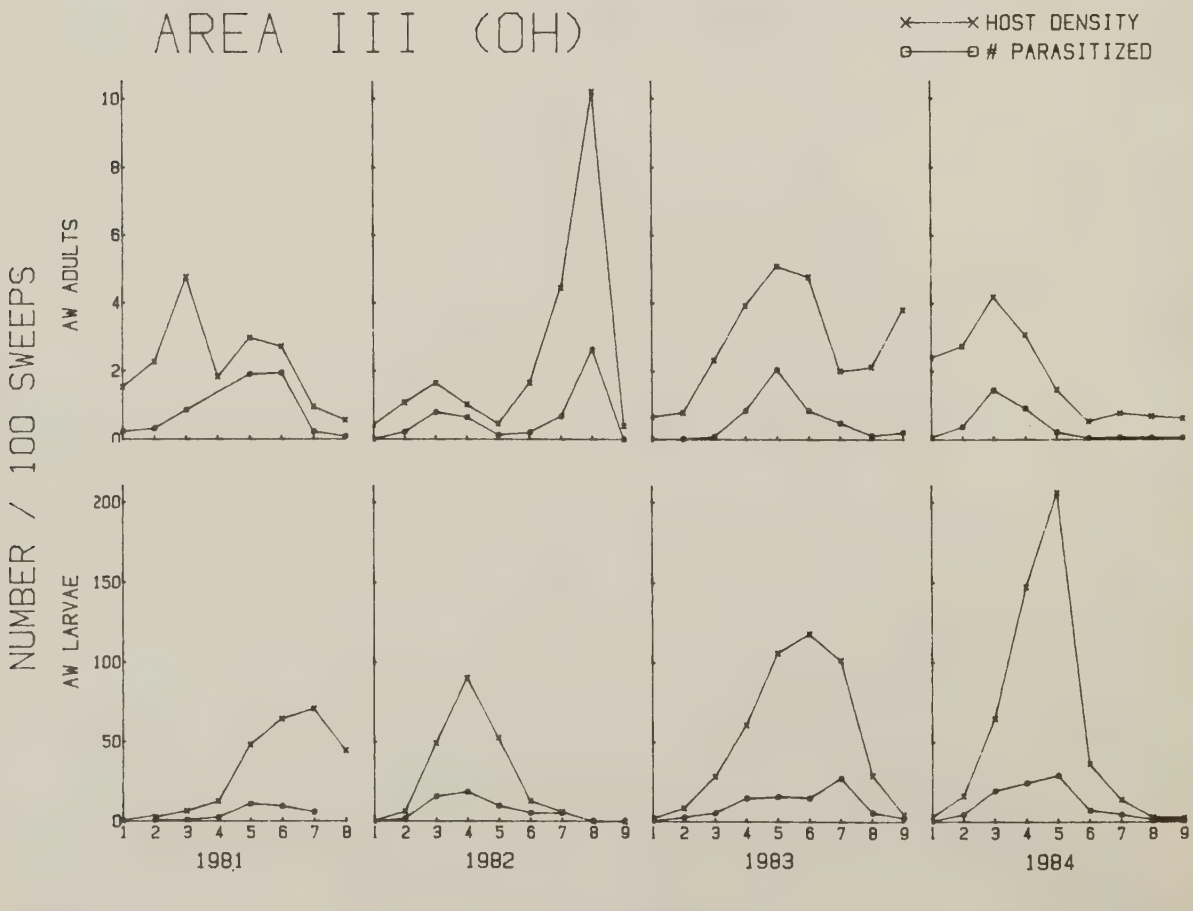


Figure 13.

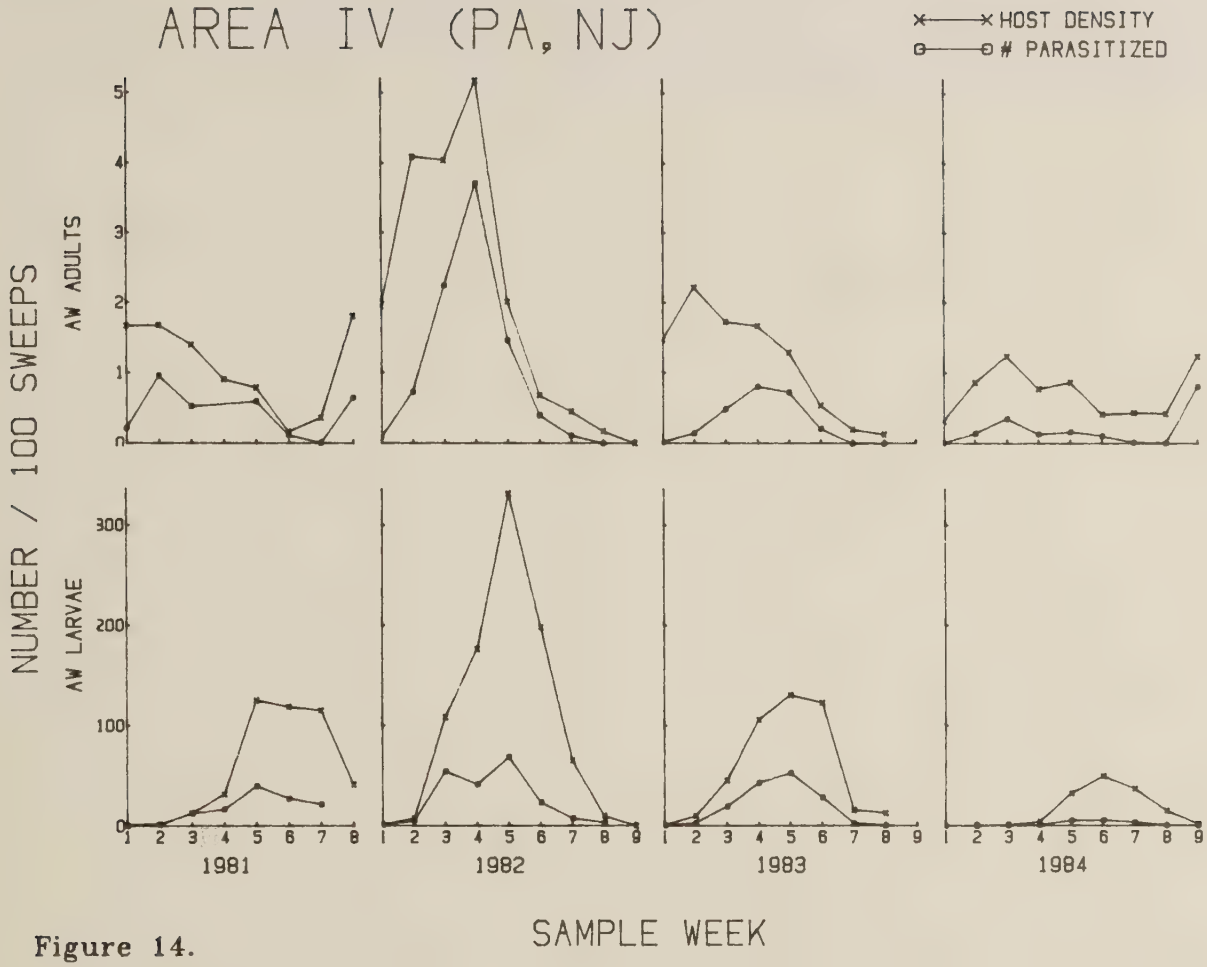

AREA IV (PA, NJ)
HOST DENSITY
PARASITIZED
NUMBER / 100 SWEEPS
AW ADULTS
AW LARVAE
1981
1982
1983
1984
SAMPLE WEEK

Figure 14.

We are continuing to monitor the economics of growing alfalfa by our approximately, 150 cooperators. This information is being compiled and analyzed by the Economic Research Service.

The Evaluation Project was originaly designed to terminate after the 1985 survey season, however, during the 1984 AW Evaluation critique meeting, it was recommended that the program be continued through 1986. This would allow us to document the continuing influx of parasitoids into the western areas, particularly Nebraska which has been surveyed for only two years. The recommendation included the possibility of discontinuing the survey, or reducing the sampling scheme in area IV (PA, NJ). This was proposed and approved by the AW Biocontrol Planning Committee in January.

Project Number: AW 2.1.1
Project Title: Alfalfa Weevil Rearing
Report Period: October 1, 1983 - September 30, 1984
Report Type: Interim
Project Leader: P.C. Kingsley

This project is ongoing and additional information will appear in the next Otis Methods Development Center Progress Report.

Project Number: AW 3.1.1
Project Title: Alfalfa Weevil Strain Identification Project: A report on its history and present status
Report Period: October 1, 1983 - September 30, 1984
Report Type: Interim
Project Leaders: Philip C. Kingsley and J. Romero-Andreas

This project, in cooperation with the University of Wisconsin, was initiated by the AW Planning Committee and the Otis Methods Development Center in the spring of 1983. Its objective was to identify and map the ranges of the two AW strains, eastern and western. These strains presumably developed from two separate introductions into the United States, one in Utah and another in Maryland, from two different locations in Eurasia. Although they are morphologically indistinguishable, the strains vary in their biology and, reproductively, are only partially compatible. At that time, there was mounting evidence from several researchers that electrophoresis might be used as a technique for their identification.

The relevance of strain identification to APHIS's AW Biocontrol Program is as follows. Alfalfa weevils are mass collected from fields in the eastern US, where parasitism rates by particular species are high. These insects are transported and released in fields further west in an effort to colonize these parasitoids. This method of distributing and establishing parasitoids was first attempted, and has since proven successful, in several midwestern and southern states. Because a small number of unparasitized weevils may survive and reproduce in the released area, some western states are reluctant, and rightly so, to use this technique if only western weevils occur there. The alternative is to establish field insectary sites in these areas and release lab-reared parasitoids. After the parasitoids have become well established, parasitized weevils can then be distributed from these sites throughout the state. Although effective, this method can be slower and more costly. If we were certain that eastern strain weevils were already present in these states, our cooperators may have opted for the parasitized host method of release.

Since releases were scheduled to begin in several of the western and south central states in fiscal 1984, we were under pressure to obtain this information quickly. Gambling that a method of identification would soon be available, we solicited help from APHIS line and their cooperators in collecting samples of adult weevils during the 1983 season from several locations in states west of the Mississippi River. With an excellent response, we collected weevils from nearly 100 sites in 19 states. Live specimens were sent to the University of Wisconsin and frozen for storage. It soon became apparent, however, that a suitable technique was not going to be developed in time by any of the researchers working on the problem. Using samples collected that summer Dr. Jeanne Romero Andreas, a post-doctoral student at the University of Wisconsin, began testing numerous enzymes. A few months later, she had found one enzyme in particular that might prove useful in strain identification. After further investigation, using a local eastern strain culture, she found that the expression of this enzyme changed with the physiological age of the adult weevil. The time in which the gene that produced this enzyme was expressed, varied with strain thus providing a method for identification. That is, western weevils showed the enzyme two to three weeks sooner than eastern weevils; so if we know that a weevil is approximately 30 days old and it shows this enzyme, it must be a western strain weevil.

Unfortunately, this technique does not lend itself to the weevils collected in 1983, since we do not know the exact age of those specimens (more about the possible use of these weevils below). Jeanne continued the search for an enzyme that would be unique to one of the strains regardless of the weevils' age or reproductive state, without success. By this time, the decisions had already been made by APHIS and their cooperators to set up insectaries in those 12 western and south central states in question. We were faced with ending the strain ID project or collecting additional samples of known age. An intermediate solution was proposed because of the considerable interest in this project from outside the APHIS program by several cooperators. The insectaries mentioned above, three to six per state, are routinely monitored by APHIS and state personnel for weevil densities and parasitism information. Samples of larvae are sent to one of three processing laboratories for rearing. Adults (normally discarded) will be collected as they emerge from these samples in the laboratory, fed for 30 days, and give us weevils of a known age suitable for electrophoresis. Samples will then be sent to the Mission Biological Control Laboratory for electrophoresis, as our cooperative agreement with the University of Wisconsin for this project expired in March. If all goes well, we should be able to determine what strain is in each of these insectaries. In addition to these insectary sites, we invite cooperators to collect samples from other areas in their states and submit them to the Mission Laboratory. Please contact me for additional information.

When it became apparent that electrophoresis might not provide us with the simple test of identity we had anticipated, we began to pursue a new taxonomic technique that utilizes cuticular hydrocarbons. This method is based on the differences between hydrocarbons washed off the insects cuticle then analyzed by gas chromatography. A unique advantage to this process is the longevity of these hydrocarbons, as specimens dead for several years can still be identified. The Gulfport National Monitoring and Residue Analysis Laboratory has been aiding us in this work and so far the results look promising, with further tests continuing. Ultimately, we hope to identify to strain, the 10,000 specimens collected in 1983 and stored at the University of Wisconsin. See Appendix 1.

Appendix 1

Age and Sex Dependent Isozyme Expression in a Wisconsin Population of Adult Alfalfa Weevils, Hypera postica (Gyllenhal) (Coleoptera: Curulionidae)

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Abstract

Polyacrylamide gel electrophoresis was used to compare isozyme expression in newly eclosed adult alfalfa weevils, Hypera postica (Gyllenhal), with isozyme expression in overwintered adults from the same generation. A sample of the subsequent generation of adults was reared in the laboratory and tested from 7 to 90 days after adult eclosion to investigate the possibility that changes in isozyme frequencies during the adult life cycle were associated with diapause, reproductive maturation, or overwintering. The frequencies of two isozymes of glucose 6-phosphate dehydrogenase (G6PDH-1 and G6PDH-2) and two isozymes of aldehyde oxidase (AO-1 and AO-2) were different in weevils of different ages. The isozyme G6PDH-2 was present in 82% to 92% of the newly eclosed weevils, whereas G6PDH-1 was present in 85% to 100% of both the overwintered weevils and the laboratory-reared weevils over 60 days old. Newly eclosed weevils expressed only AO-2, whereas weevils just entering diapause expressed AO-1 and AO-2 simultaneously. Laboratory-reared weevils over 60 days old expressed only AO-1, but AO-2 was present in 91% of the overwintered males and 100% of the overwintered females. Expression of an isozyme of lactate dehydrogenase (LDH-H) was detected in all male weevils over 60 days old, whether laboratory-reared or overwintered. No LDH-H was detected in young males or female weevils of any age.

The adult form of the eastern strain of the alfalfa weevil, Hypera postica (Gyllenhal), experiences significant physiological and environmental changes during its lifetime. Adult diapause begins a few weeks to a month after eclosion in the spring (Litsinger and Apple, 1983). The weevils stop feeding, migrate to areas adjacent to the field, and form quiesent aggregations under leaf litter (Prokopy et al., 1967; Guerra and Bishop, 1962). Adults in diapause exhibit a strong negative phototaxis, reduced oxygen consumption, hypertropy of the fat bodies, and a lack of ovarian and testicular development (Pienkowski, 1976; Litsinger and Apple, 1973). In September and October, the weevils return to the field, resume feeding, and complete reproductive development (Litsinger and Apple, 1973). Male and female reproductive organs do not regress during the winter, and mating and oviposition occur, whenever temperatures permit, until late spring (Tombes, 1964; Campbell et al., 1961).

The profound physiological changes associated with adult diapause and the resumption of reproductive development, and the quiesence induced by cold winter weather might be expected to be accompanied by changes in isozyme expression. A preliminary investigation, done as a part of another study, revealed that field collections which included a mixture of overwintered and newly eclosed alfalfa weevil adults showed different isozyme frequencies for glucose 6-phosphate dehydrogenase (G6PDH), aldehyde oxidase (AO), and lactate dehydrogenase (LDH) than field collections composed only of newly eclosed adults. In this study we used polyacrylamide gel electrophoresis to examine G6PDH, AO, and LDH isozyme frequencies in newly eclosed adults, overwintered adults from the same generation, and laboratory-reared adults which developed from eggs laid by the overwintered adults. The laboratory-reared adults were periodically sampled between 7 and 90 days after adult eclosion to determine when the transition in isozyme expression occurred, and if a shift in expression was associated with diapause or reproductive maturation in either sex.

Materials and Methods

Weevil collection and Rearing

Material for electrophoretic analysis was obtained from three separate collections which were taken in an alfalfa field near Madison, Wisconsin. Collection 1: Fifth instar weevil larvae were collected June 10, 1983, and reared to the adult stage in the laboratory. Newly eclosed adults were removed July 21 and frozen at -90°C until electrophoretic analysis. Collection 2: Adult weevils were collected May 1, 1984, and maintained in the laboratory until electrophoretic analysis, several days to a week after collection. Examination of individual specimens revealed the darkened elytra and somewhat ragged appearance characteristic of overwintered weevils. None of the specimens collected had the light brown or grayish elytra with the sharply delineated dorsal stripe which is characteristic of newly eclosed adults. Also, on May 1, larvae in the field population had not yet begun pupating. We therefore concluded that this collection was composed entirely of overwintered adults. That being the case, Collections 1 and 2 comprosed samples from a single weevil generation. Collection 3: Fifth instar larvae were collected June 1, 1984, and reared to adults in the laboratory. Newly eclosed adults were removed June 12 and placed in one gallon (3.8 liter) ice cream cartons in a growth chamber at constant 20°C and 14h photophase. Fresh alfalfa was provided daily for 30 days. Surviving weevils were then moved to clean cartons, each of which contained shredded paper towels and a 5 ml glass vial filled with a 2% (w/v) sucrose solution and plugged with a cotton wick. The paper towels were lightly misted with water 2 to 3 times per week. The photophase was reduced to 13h and the night temperature reduced to 14°C when the weevils were 40 days old. Alfalfa was offered again daily from 55 days after eclosion. At 64 days after eclosion, when feeding was first evident, the weevils were transferred to clean cartons which were provided daily with fresh alfalfa. The photophase was reduced to 12h and the night temperature reduced to 12°C 80 days after eclosion. Samples of adults from this collection were removed from the cartons at 7, 21, 30, 60, and 90 days after eclosion for electrophoretic analysis.

Living or frozen insects were placed on a covered ice block, and males were initially separated from females using the criteria of smaller size and the presence of an abdominal concavity. The abdomen of each weevil was then opened and the sex comfirmed by examining the genitalia. The partially dissected weevils were immediately placed in Tris-citrate buffer and processed for electrophoresis as described below.

Electrophoresis

Crude protein extracts were prepared by homogenizing individual insects in 0.06 ml of chilled Tris-citrate buffer (pH 8.2). The homogenate was centrifuged, and 0.04 ml of supernatant was added to 0.04 ml of a Tris-citrate buffer containing 30% (v/v) glycerine and 0.05% (w/v) bromephenol blue. Isozymes were separated by applying 0.015 ml of the diluted protein extract to a vertical polyacrylamide gel system. The discontinuous gel system originally described by Ornstein (1964) and Davis (1964) was modified for use in slab gel units and all electrophoresis was performed at $4^{o}C$. The stacking gel formulation was modified from that of Laemmli (1970) to permit nondenaturing electrophoresis without photopolymerization.

The enzymes investigated in this study were aldehyde oxidase (AO), glucose 6-phosphate dehydrogenase (G6PDH), and lactate dehydrogenase (LDH). The enzyme staining techniques of Bush and Huettel (1972) were used without modification. Preliminary experiments had revealed that freezing intact, live weevils had no effect on enzyme mobility or apparent activity for periods up to one year, provided that the insects remained frozen until immediately before processing.

Results

Differential isozyme expression was observed in the newly eclosed and overwintered weevils from the first and second collections, respectively (Table 1, Figure 1). A majority of the newly eclosed weevils expressed G6PDH-2, whereas most of the overwintered weevils expressed G6PDH-1. The isozyme AO-1 was not observed in the newly eclosed weevils, whereas a majority of the overwintered weevils expressed AO-1 and AO-2 simultaneously. The isozyme AO-1 was present in all overwintered males, either alone or in conjuction with AO-2. However, AO-1 was not found in 23% of overwintered females. Faint LDH bands were occasionally observed in both sexes in newly eclosed weevils, but all of the overwintered male weevils showed a poorly resolved by heavily stained LDH (LDH-H) not found in the younger males or females from either sample (Fig. 1).

The third collection of weevils was reared in the laboratory under a regime of decreasing photophase and night temperatures. Within four weeks after eclosion, the weevils began to exhibit behavior typical of diapause; i.e., weak attempts at flight followed by a cessation of feeding, a negative phototaxis, the formation of aggregations, and quiesence. Subsequently, light feeding, mating, and egg masses were first observed 64, 87, and 93 days after eclosion, respectively.

In these weevils, the transition from G6PDH-2 to G6PDH-1 occurred between 40 and 60 days after eclosion, when the weevils still appeared to be in diapause (Table 2). The relative frequencies of G6PDH isozymes in 7 and 90 day old weevils were comparable to the frequencies found in the newly eclosed and overwintered weevils from the previous generation (cf. Table 1). Between 5% and 13% of the male weevils did not express G6PDH-2 at 7, 21, and 30 days, and 3% to 5% of the females did not express G6PDH-1 at 60 and 90 days. These data suggest that some weevils may lack the capacity for expression of one or the other of the G6PDH isozymes.

The expression of the aldehyde oxidase isozymes shifted from AO-2 to AO-1 between 21 and 60 days (Table 2). Although both isozymes were expressed simultaneously in 30 day old weevils, AO-2 was no longer detectable in 60 and 90 day old weevils. The presence of AO-2 in overwintered weevils (Table 1) suggests that the expression of AO enzymes may be influenced by multiple factors, including diapause and sex.

The LDH-H phenotype was limited to males and occurred in all males tested on or after 60 days following eclosion (Table 2) and in all overwintered males (Table 1). Although attempts to resolve LDH-H into distinct bands by varying electrophoretic and extraction conditions failed, the LDH-H phenotype was invariable in appearance when present, repeatable, and quite distinct from the female phenotype (Fig. 1). These data suggest that the presence of LDH-H may be associated with the development and maintenance of male reproductive capability.

Discussion

This study was conducted as a part of a larger study on isozyme frequencies in the eastern and western strains of the alfalfa weevil. Sell, et al. (1978) used starch gel electrophoresis to detect strain-specific differences in isozyme frequencies of overwintered alfalfa weevils. Our attempts to enlarge upon this work, using weevils in adult diapause, failed until we abandoned starch gel electrophoresis for the discontinuous polyacrylamide gel system described in Materials and Methods. Subsequent screening of both laboratory-reared and field-collected weevil adults indicated that isozyme frequencies for G6PDH, AO, and LDH were not in accordance with Hardy-Weinberg expectations for simply inherited phenotypes in a randomly breeding, diploid population. A closer examination of the data revealed that electrophoretic variability for these three enzymes was detected in field-collected insects which included a mixture of overwintered and newly eclosed adults. The isozyme frequencies discovered could not then be ascribed to allalic differences unless developmental and environmental effects were controlled.

The design of the experiments in this study permitted the comparison of isozyme frequencies in newly eclosed weevils, adults in diapause, and reproductive adults. The expression of G6PDH shifted from G6PDH-2 to G6PDH-1 toward the end of diapause in the laboratory-reared adults. The frequency of G6PDH-1 in 90 day old adults was not different than the frequency of G6PDH-1 in overwintered adults. This result suggested that the shift in G6PDH isozyme frequency may be associated with reproductive maturation rather than a change in environmental conditions. A small but significant number of young weevils did not appear to express G6PDH-2, and 3% to 5% of the reproductive females did not appear to express G6PDH-1. The 7-30 day old weevils which expressed G6PDH-1 rather than G6PDH-2 might subsequently undergo diapause and normal reproductive development while lacking the genetic capacity to express the G6PDH enzyme normally associated with a given developmental stage. As eggs were not present in 7-30 day old females expressing G6PDH-1, the expression of G6PDH-1 was probably not a result of reproductive maturation, but merely associated with it in most cases.

The pattern of expression of AO isozymes was somewhat different than the pattern observed for G6PDH isozymes. The isozyme AO-2 was observed in all of the newly eclosed weevils tested from both collections, whereas AO-1 was not present. In the developmental study, a transition from AO-2 to AO-1 began to occur between 21 and 30 days after eclosion. Between 96% and 100% of the 30 day old weevils expressed AO-1 and AO-2 simultaneously, but AO-2 was no longer present 60 days after eclosion. Although AO-2 was not found in the reproductive weevils in the developmental study, most overwintered weevils expressed AO-2 either alone or in conjuction with AO-1. The expression of AO-2 may be induced in the reproductive adult when feeding is resumed in the spring after a prolonged period of inactivity.

The LDH-H data were unambiguous. None of the 290 females tested in this study and none of the males 30 days old or younger expressed LDH-H, whereas LDH-H was present in all of the males 60 days and older, including overwintered males. Electrophoretic screening, done as a part of another study, has shown that reproductive males maintained at 20°C in laboratory culture for 9 months continue to express LDH-H (unpublished data). The specificity and stability of LDH-H expression suggests that the appearance of LDH-H may be correlated with testicular development. In postpubertal mammals and some birds, the isozyme LDH-X appears in primary spermatocytes, but in no other male tissue, and is absent in females (Wheat and Goldberg, 1975).

It is often implicitly assumed that an electrophoretic phenotype (an "isozyme" in most cases) remains constant throughout a given life stage of an individual insect. In a recent review of insect molecular systematics, Berlocher (1984) emphasized the importance of determining the effect of environment on isozyme expression. In this study, we have shown that developmental age and sex, as well as environmental conditions, can profoundly influence isozyme expression. Thus, differences in isozyme frequencies among insect strains or races may not be an accurate indicator of genetic distance unless the age of specimens is controlled and sex differences are accounted for. The need to examine age effects may be particularly important in those insect species in which an adult diapause occurs and overlap of generations is possible.

It is also frequently assumed that the electrophoretic variants of a given enzyme are alleles at a single locus. The AO data demonstrate that the presence of a composite phenotype does not necessarily indicate that the isozymes which compose the phenotype are allelic. The AO-1+2 phenotype was due not to heterozygosity, but to simultaneous expression of two different aldehyde oxidases. In cases in which lack of Hardy-Weinberg equilibrium is found to occur, the underlying cause of the apparent disequilibrium may be nonallelic genetic variation.

Acknowledgment

The authors wish to thank Larry Pfiel for technical assistance, Walter Gould for assistance with weevil collection and maintenance, and Jane Harrington and Walter Goodman for reviewing the manuscript. This research was supported by the College of Agricultural and Life Sciences, University of Winsconsin-Madison, and by USDA Cooperative Agreement 12-16-81-008.

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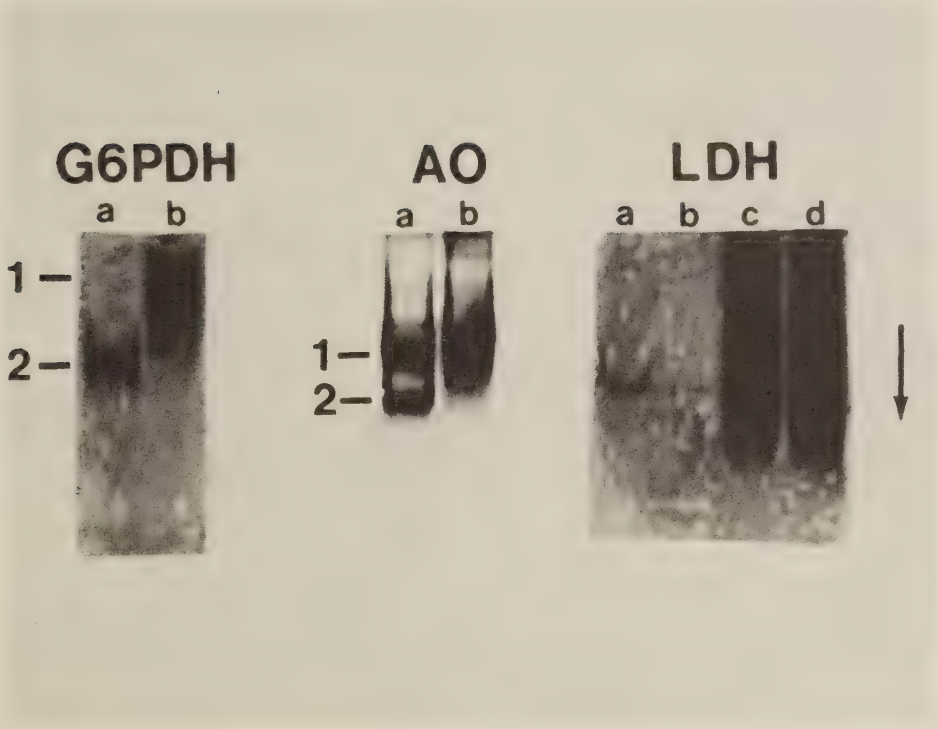
Table 1. Isozyme frequencies for G6PDH-1, G6PDH-2, AO-1, AO-2 and the presence (+) or absence (-) of LDH-H in adult alfalfa weevils of different ages.

		Isozyme Frequencies							
		G6PDH		AO			LDH-H		
Age	Sex	1	2	1	2	1+2	+	-	Total No. Tested
Newly eclosed	M	.18	.82	.00	1.00	.00	.00	1.00	45
	F	.07	.93	.00	1.00	.00	.00	1.00	45
Overwintered	M	.94	.06	.09	.00	.91	1.00	.00	78
	F	.85	.15	.00	.23	.77	.00	1.00	101

Table 2. Isozyme frequencies for G6PDH-1, G6PDH-2, AO-1, AO-, and the presence (+) or absence (-) of LDH-H in male and female alfalfa weevils 7 to 90 days after adult eclosion.

Days from Eclosion	Sex	Isozyme Frequencies							Total No. Tested
		G6PDH		AO			LDH-H		
		1	2	1	2	1+2	+	-	
7	M	.13	.87	.00	1.00	.00	.00	1.00	37
	F	.08	.92	.00	1.00	.00	.00	1.00	37
21	M	.05	.95	.00	.87	.13	.00	1.00	20
	F	.00	1.00	.00	1.00	.00	.00	1.00	20
30	M	.12	.88	.04	.00	.96	.00	1.00	25
	F	.08	.92	.00	.00	1.00	.00	1.00	25
60	M	1.00	.00	1.00	.00	.00	1.00	.00	36
	F	.97	.03	1.00	.00	.00	.00	1.00	32
90	M	1.00	.00	1.00	.00	.00	1.00	.00	20
	F	.95	.05	1.00	.00	.00	.00	1.00	20

Figure 1. Nondenaturing polyacrylamide gels of G6PDH AO and LDH in adult male and female alfalfa weevils of different ages. G6PDH a) newly eclosed female; b) overwintered male. AO a) overwintered female; b) overwintered male. LDH a) newly eclosed male; b) female 60 days old; c) male 60 days old; d) overwintered male. Note intense staining of LDH-H in c and d. Arrow indicates direction of protein migration.



Project Number:	MBB 1.1.4
Project Title:	Direct Release of *Pediobius foveolatus* into Soybean Fields for Control of Mexican Bean Beetle
Report Period:	October 1, 1983 - September 30, 1984
Report Type:	Interim
Project Leader:	O.T. Forrester

This project is ongoing and additional information will appear in the next Otis Methods Development Center Progress Report.

Project Number: MBB 4.1.1
Project Title: Biological Control of Mexican Bean Beetle (MBB) Over Large Areas With Pediobius foveolatus (Pf).
Report Period: October 1, 1983 - September 30. 1984
Report Type: Interim
Project Leader: O.T. Forrester

A field study was conducted on a small truck farm in Massachusetts, during 1981 and 1982, using a direct field release of Pf to control MBB. On June 27, 1981, a release of 16,000 Pf was made; this did not provide foliage protection and chemical control was required. However, later planted beans did not require additional control measures. By early September, 95.5 percent of the MBB larvae collected were parasitized. MBB populations in 1982 were low; a release of 500 Pf was made in a localized infestation. No chemical controls were required in 1982 or 1983. These results suggested that dramatic parasitization late in the 1981 season effected reduced populations the following year.

Field studies conducted in Ohio during 1982 showed very high parasitism late in the season 1.5 miles from parasite release points. This parasitization was of little benefit in the season of release because it occurred too late to prevent MBB defoliation. However, it was thought that Pf use may produce maximum benefits the following year.

The objective of this study was to test the hypothesis that high parasitism rates result in lower overwintering Mexican bean beetle populations and reduce the infestation levels the following year.

Study sites (treatment and control) of 36 sq. miles each were selected in areas of Ross, Pike, Scioto, Clark, and Preble Counties, Ohio with a past history of Mexican bean beetle damage in soybeans. Standard 50' x 100' nurse plots were planted on a two mile grid in the treatment areas with a mixture of 1/3 soybeans and 2/3 snap beans (fig. 1). Nurse plots were not planted in the control areas.

Overwintered MBB were attracted to the nurse plots and the resulting first generation larval populations were assessed weekly. Pf were released in the nurse plots when 50% of the MBB larvae were second instar or older. Nurse plot assessment was continued for 3 weeks after Pf release.

Soybean fields for weekly monitoring were randomly selected. Field sampling was accomplished by sampling 5 points across the field in the shape of an M. Two types of samples were taken: random (tossing a yard stick in two locations at each point) and selected (tossing a yard stick into an area with MBB life stages or feeding damage). Each plant in the 3 foot sample unit was thoroughly checked for MBB life stages and parasitized larvae. Percent defoliation was estimated to the nearest 5% and recorded as total defoliation and percent caused by MBB.

Weather was a major factor in this season's work. Nurse plot planting was delayed because of cold, wet weather. Planting began on April 26 and was completed June 3 - three weeks later than planned. Herbicide carry-over and the cold, wet weather caused snap bean mortality and several nurse plots had to be replanted.

The cold, wet spring was followed by a hot dry summer, all of which was detrimental to the development of high MBB population.

The MBB populations, in general, were low, and the economic threshold was not exceeded in any of the areas. The first generation larval peak occurred the third week of July. The second generation larval peak occurred between the third and fourth week of August. The first generation adult peak occurred during the third week of August. The second adult generation population was trending up during the last two weeks of September. MBB populations for 1984 season are plotted in Figures 2 - 6.

Nurse plot statistics are summarized in Table 1. Host counts at time of Pf release were highest in the southern-most study site, and decreased to the lowest in the northern-most study site. Nurse plot planting dates were spread over a 6 week period and Pf release occurred over a 2.5 week period.

MBB populations in Scioto, Pike, Ross, Clark and Preble Counties are plotted by weeks in Figures 3-5. Host populations were lower than desirable and economic threshold was not exceeded in any of the study areas. Plots of adult populations do not look favorable for the stated hypothesis of lowering overwintering adult populations.

Table 2 summarizes the number of soybean fields sampled in the study area, and number of fields with host material and Pf. This can be used as a measure of Pf dispersal from the nurse plots. Dispersal from the Scioto County area was to 100% of the fields checked. Pf was detected 14 miles northeast of the Scioto County study site (in the control area in Pike county) 6 weeks after Pf release.

Percent parasitized MBB for Scioto, Pike, Ross, Clark and Preble is plotted by week in Figures 7-10. Parasitization was calculated by combining random and selected samples. Parasitism rates were very high late in the season. This is in agreement with previous work in this area.

Conclusions about the effectiveness of this project cannot be made until next season. Bean leaf beetle is increasing in importance in the Scioto River Valley and all control measures were directed toward this pest.

Table 1. Summary of MBB densities, Pf releases and mummy counts in the Ohio MBB Project nurse plots. 1984.

Study Area	Size Sq. Miles	Number of Nurse Plots	Mean Counts		
			MBB Release Counts (15' of row)	No. Pf released	Peak Mummy Count (15' of row)
Clark	36	9	30	18,000	7
Clark Control	36	0	---	----	----
Preble	36	9	57	18,000	2.4
Preble Control	36	0	---	----	----
Ross	36*	5	198	25,000	11.0
Pike Control	36*	0	---	----	----
Scioto	36*	3	250	25,000	57

* Scioto River Valley

Table 2. Summary of the number of fields in study areas in which MBB and/or Pf were observed. 1984.

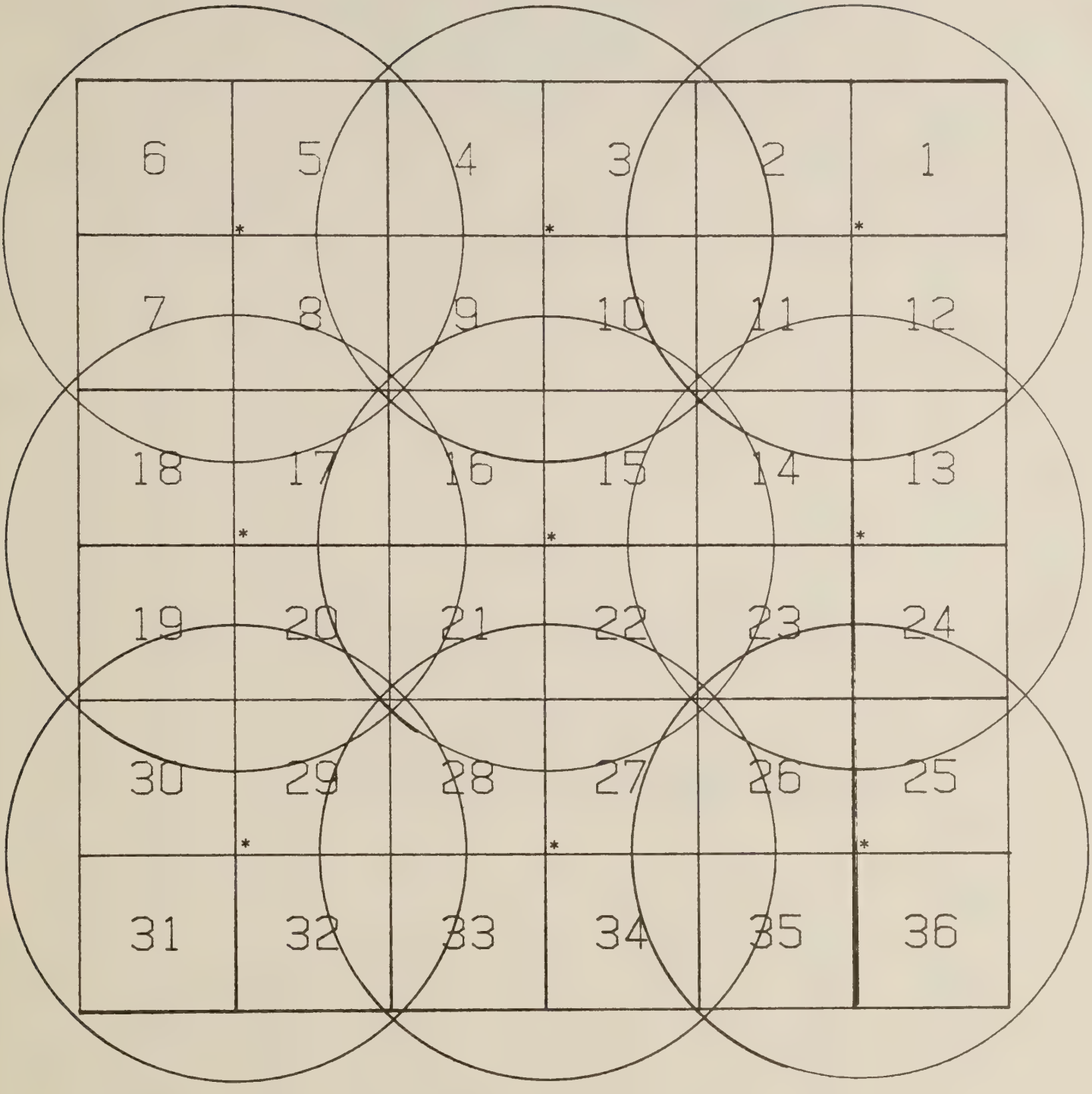
Study Area	Number of Fields Sampled	Fields with MBB	Fields with Pf
Clark	36	1-23**	1
Clark Control	36	4	0
Preble	34	28	18
Preble Control	32	12	0
Ross	17*	12	7
Pike Control	14*	13	4
Scioto	16*	16	16

* Scioto River Valley

** Only egg clusters were observed at this site and they may have been of a predaceous Coccinellid common to the area.

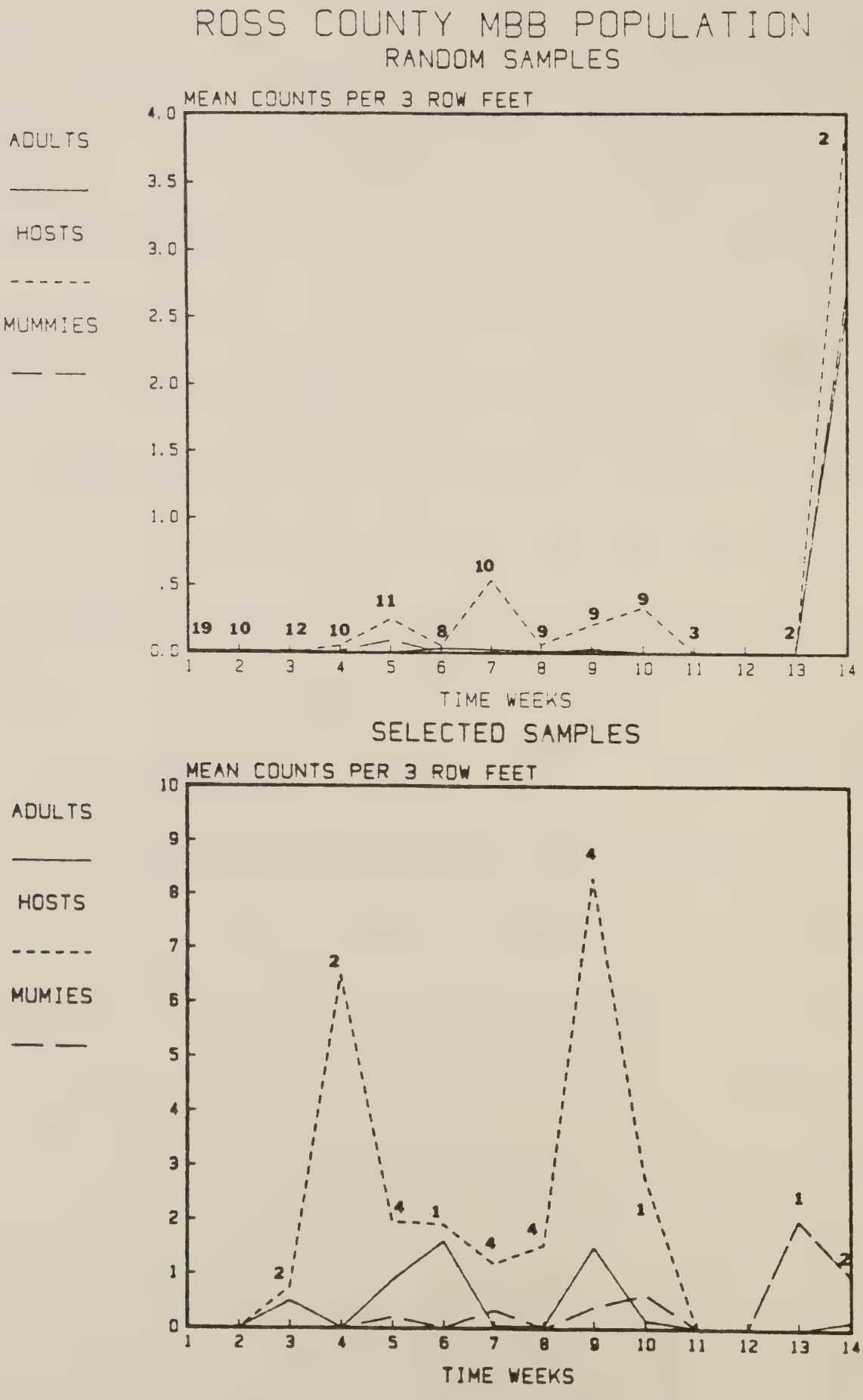
Figure 1.

STUDY SITE OF ONE TOWNSHIP



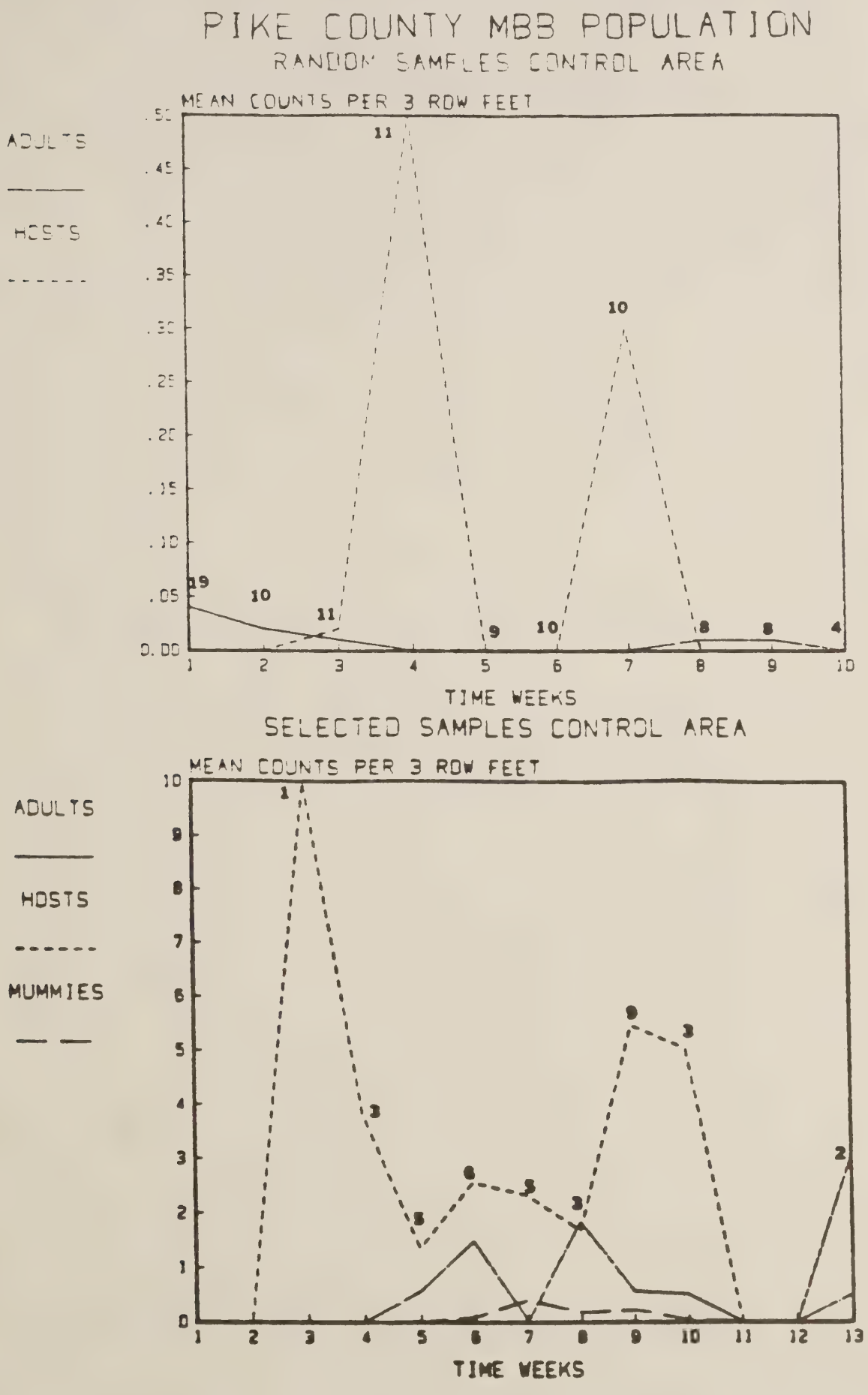
* Nurse Plot Location

Figure 2. Seasonal plot of MBB density during 1984 growing season.



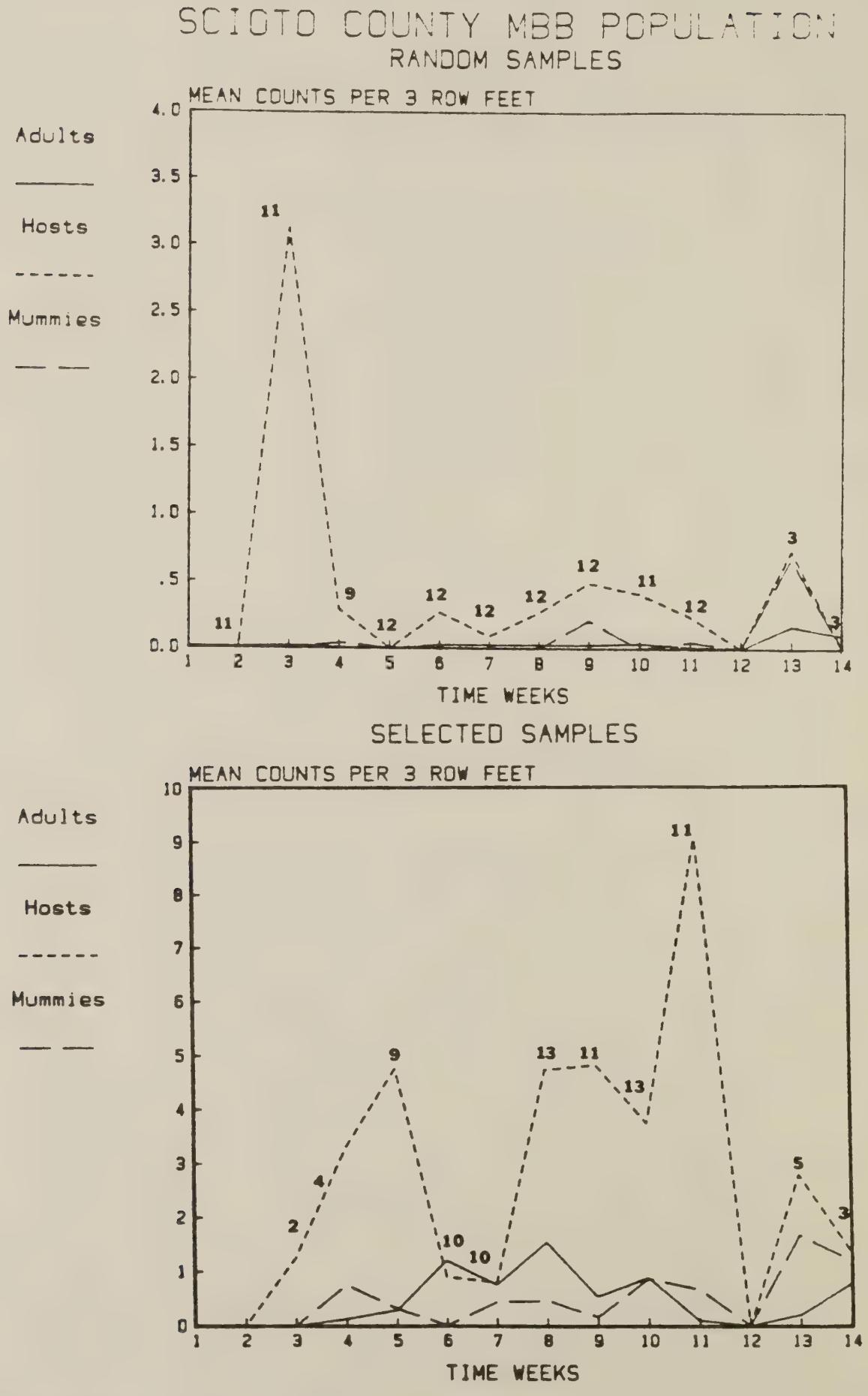
N = Number of fields sampled.

Figure 3. Seasonal plot of MBB density during 1984 growing season.



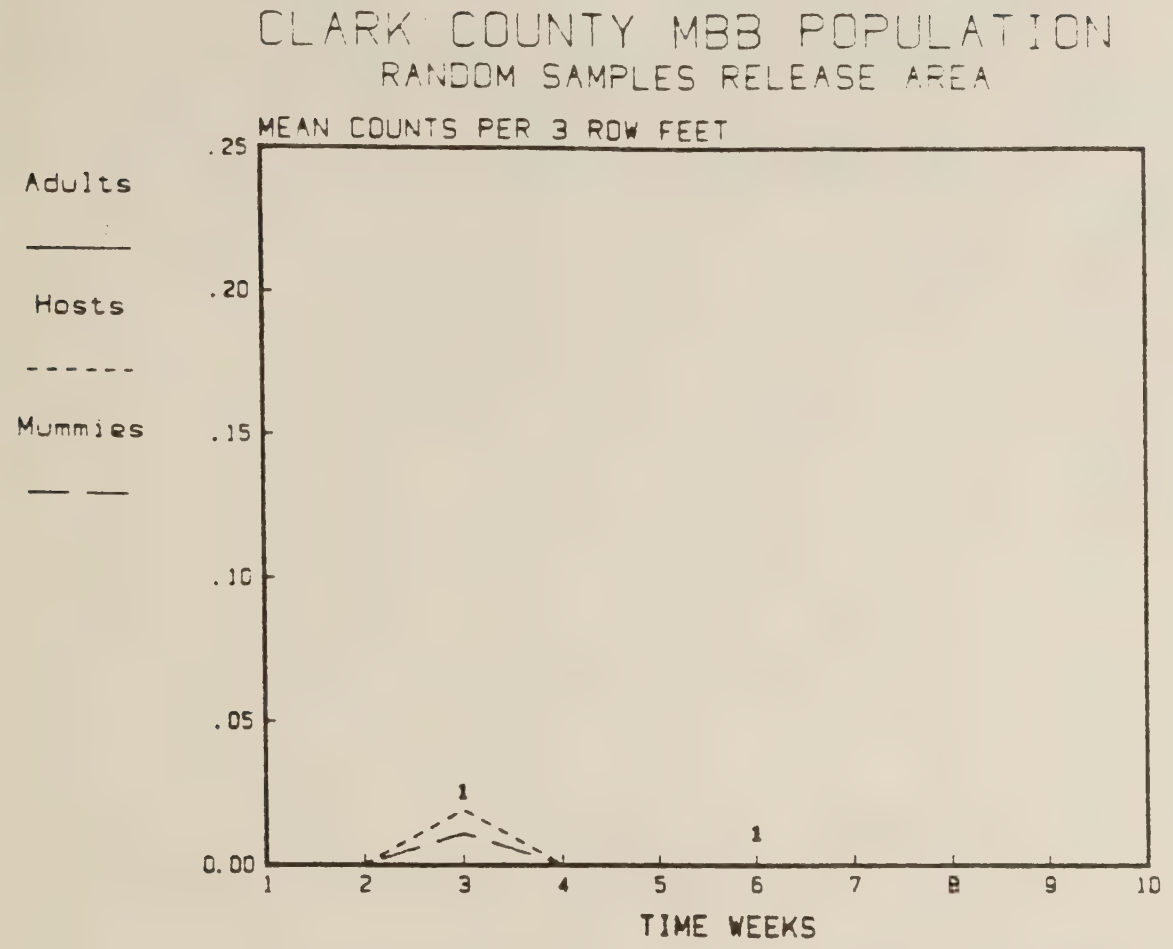
N = Number of fields sampled.

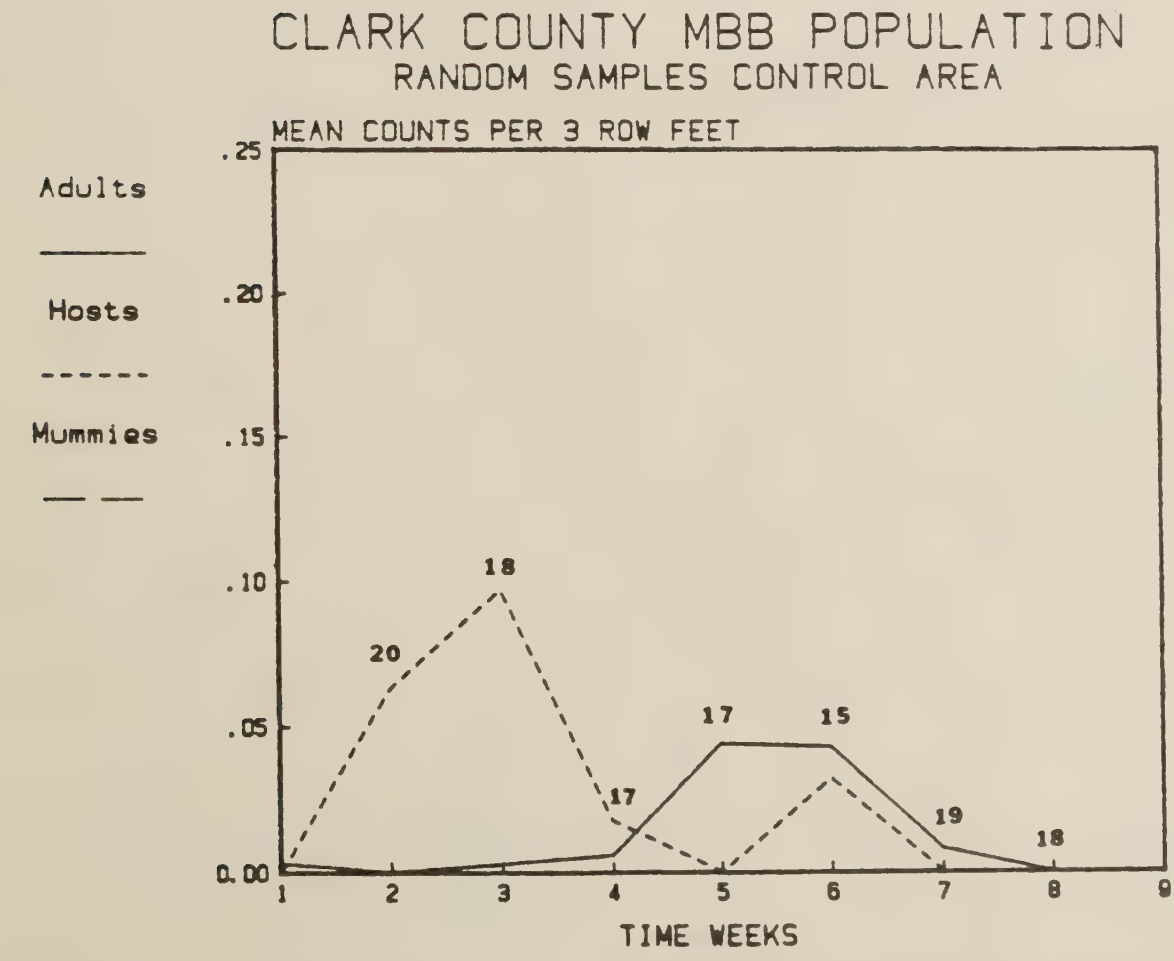
Figure 4. Seasonal plot of MBB density during 1984.



N = Number of fields sampled.

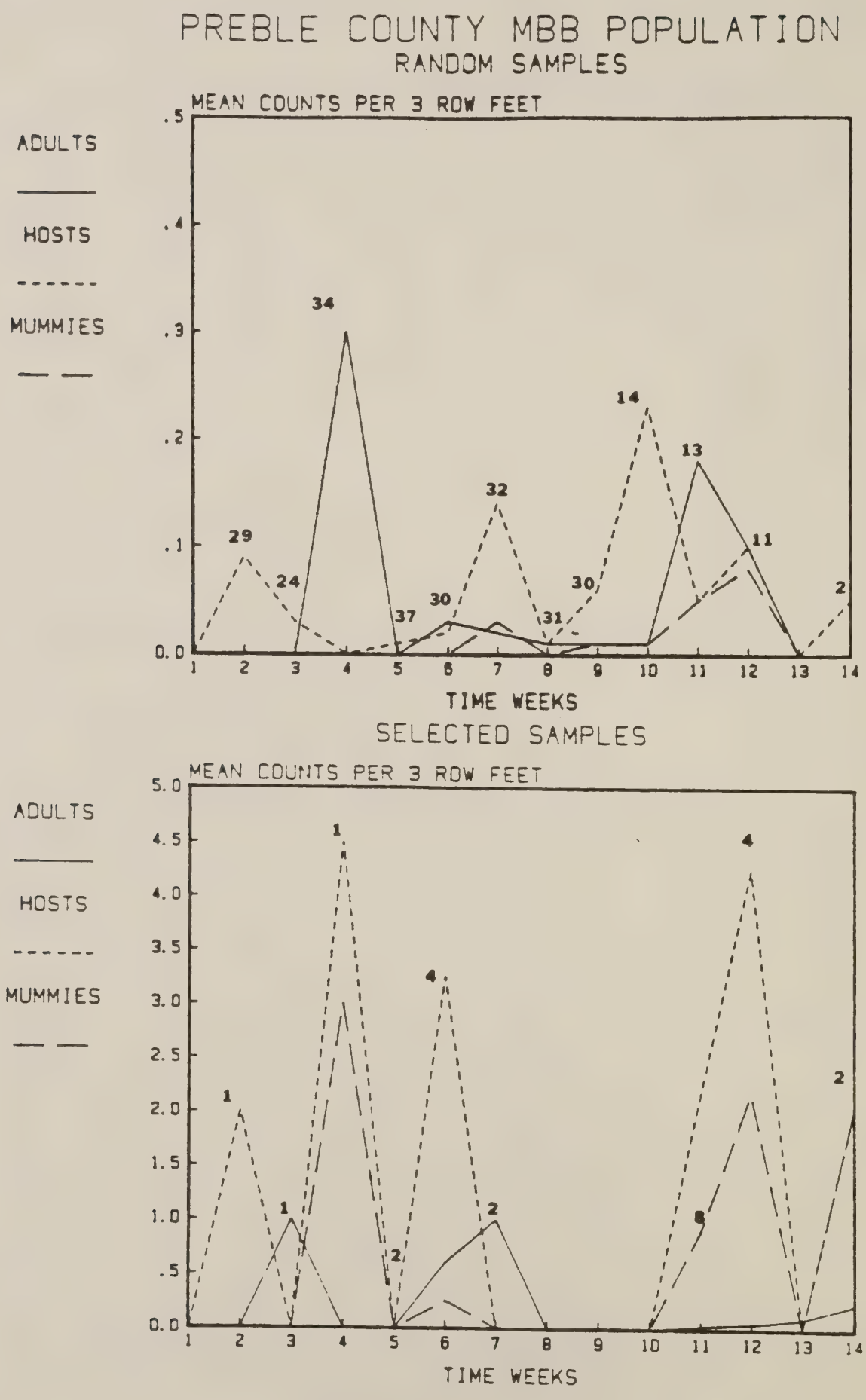
Figure 5. Seasonal plot of MBB density in 1984.





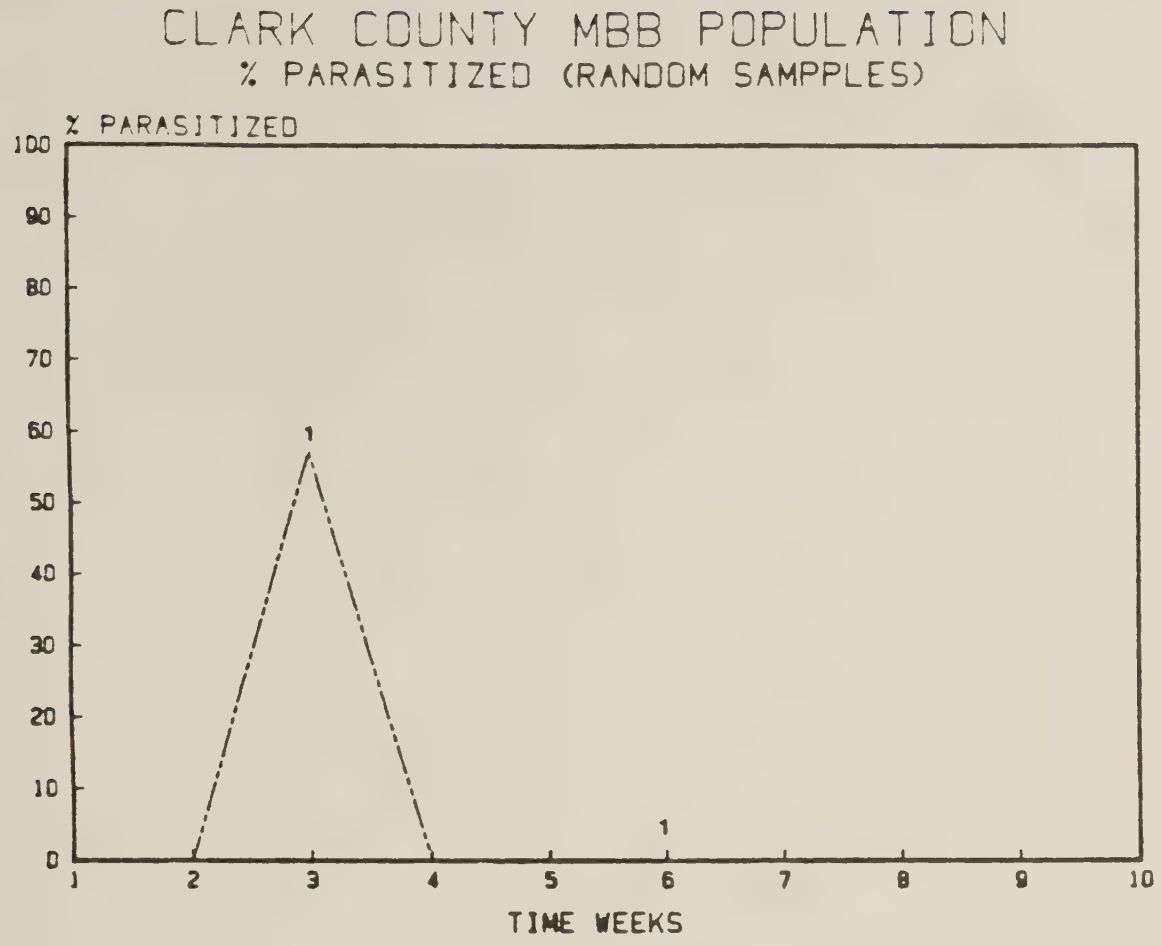
N = Number of fields sampled.

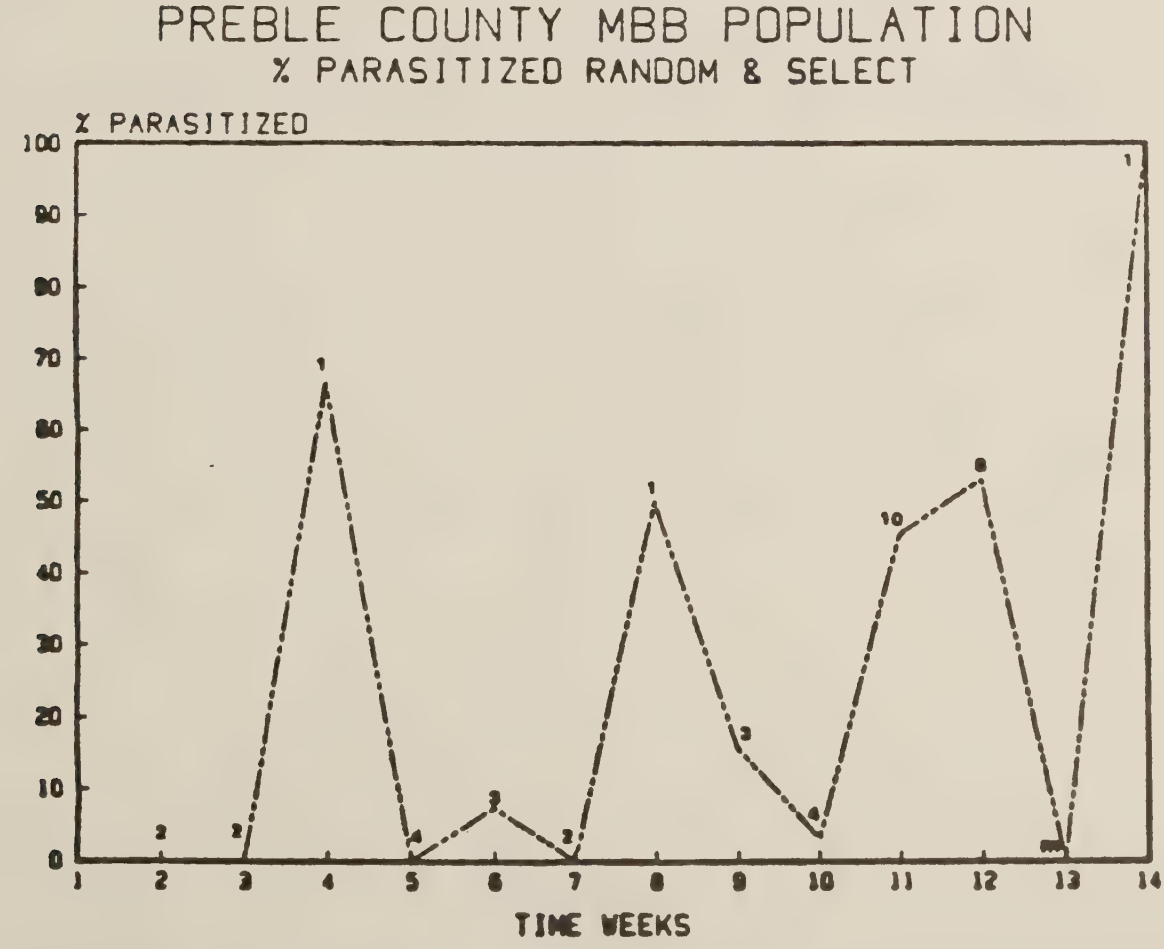
Figure 6. Seasonal plot of MBB density, 1984.



N = Number of fields checked.

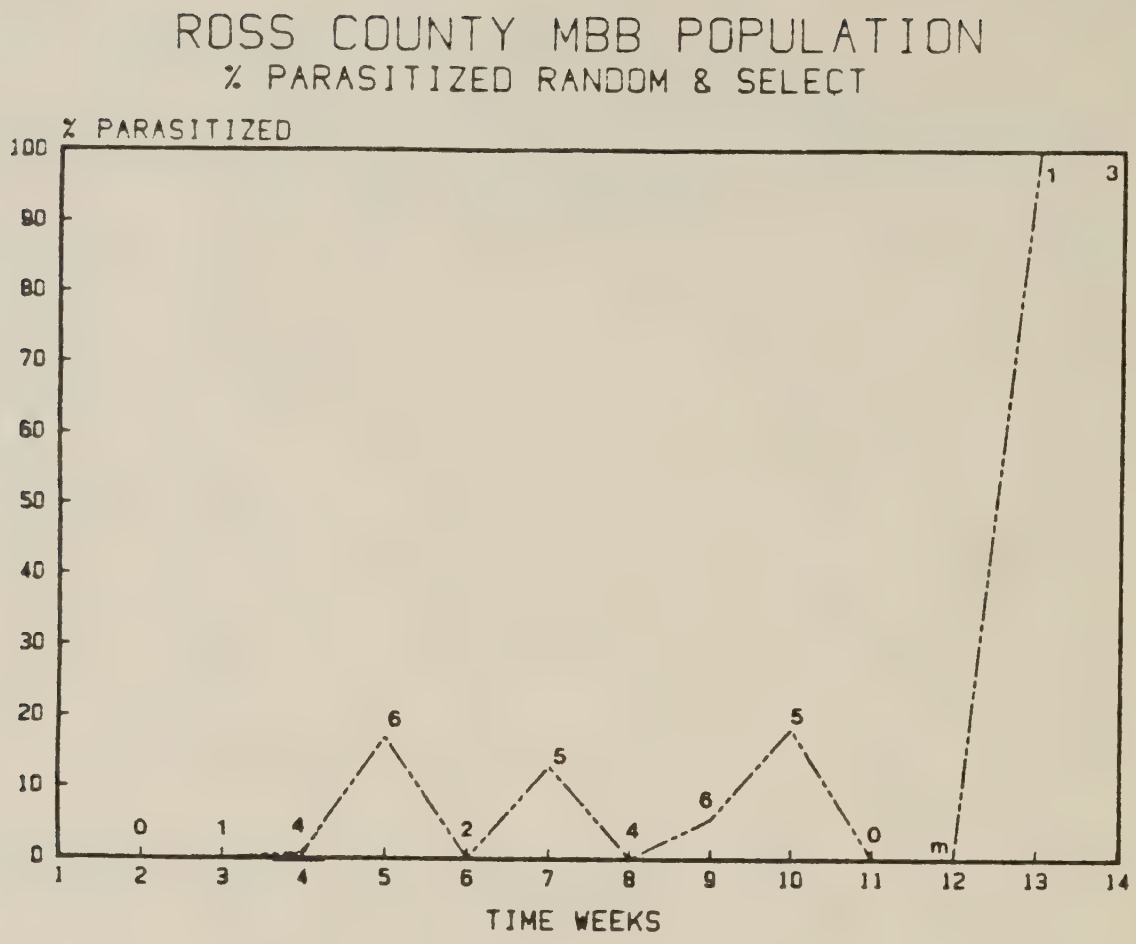
Figure 8.

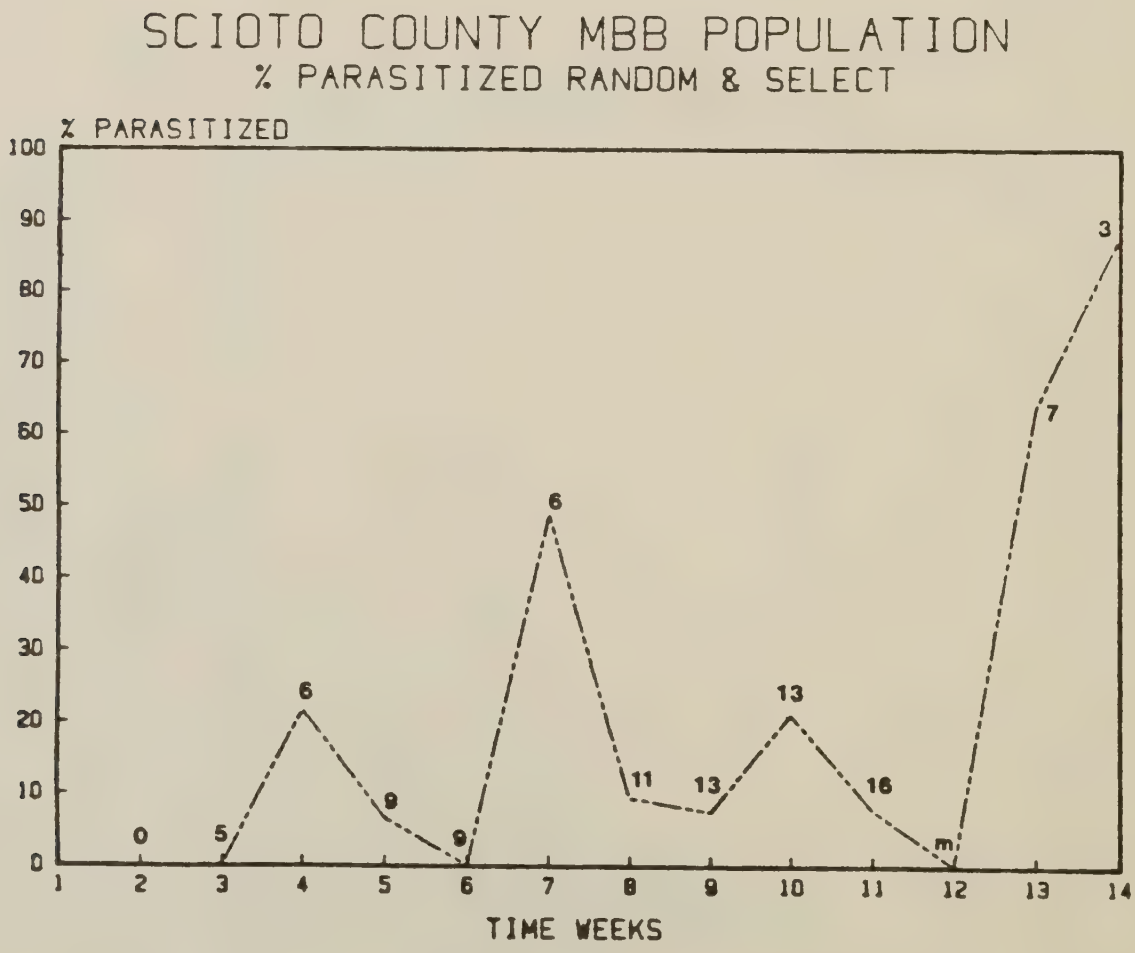




N = Number of soybean fields with host material (larvae and pupae).

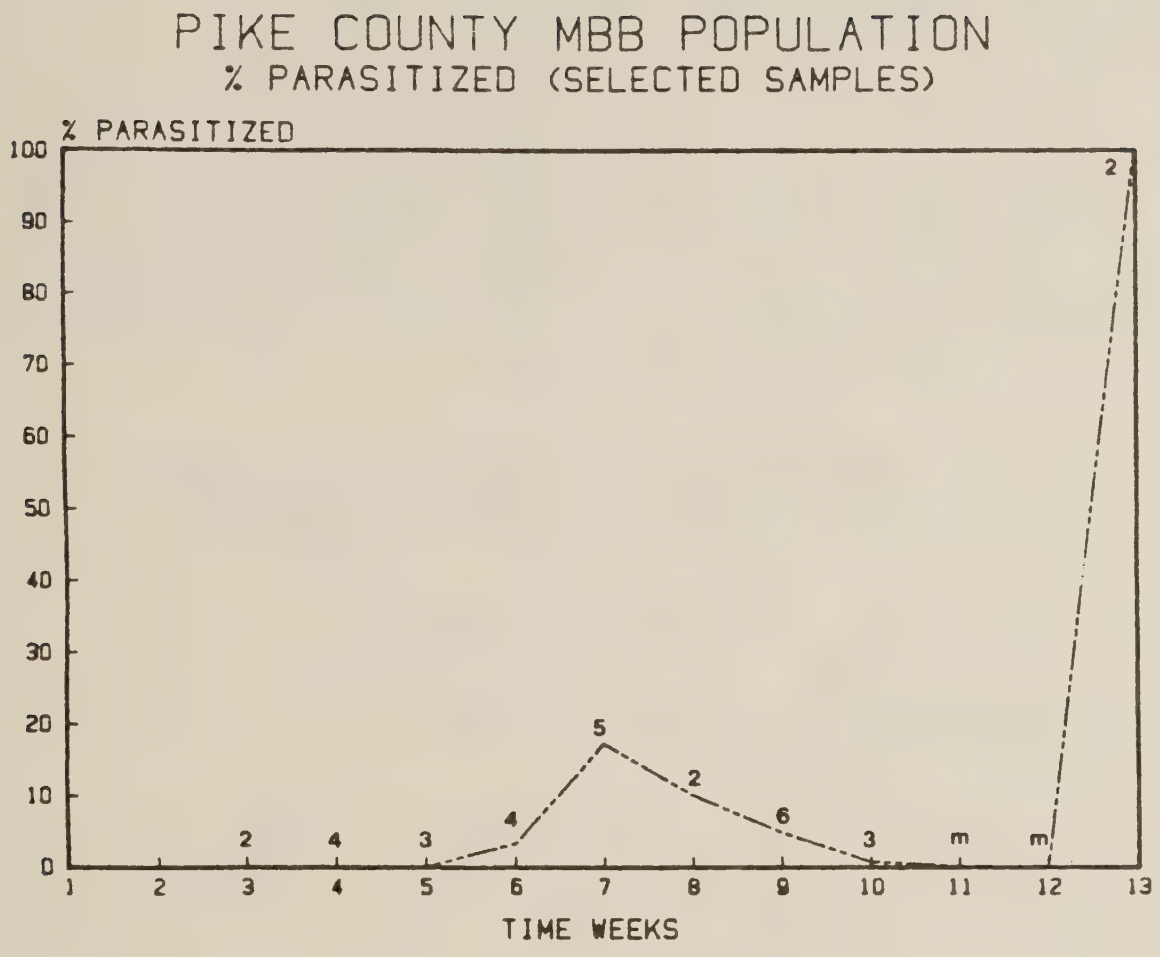
Figure 7.





N = Number of soybean fields with host material (larvae and pupae).

Figure 9.



N = Number of soybean fields with host material (larvae and pupae).

Project Number: JB 4.1.1
Project Title: Evaluation of Traps and Other Techniques for Controlling Japanese Beetles in and Around Airports
Report Period: October 1, 1983 - September 30, 1984
Report Type: Interim
Project Leaders: W. H. McLane and J. A. Finney

Japanese beetle infestations are known to occur at nearly all of the major airports in the eastern United States and from time to time adult populations are high enough to warrant regulation of airports. Adult beetles are attracted to aircraft and can easily hitchhike to western states and establish new infestations. For example, aircraft inspections in California have resulted in beetle finds on a number of planes that originated from infested airports in the east.

Infestations can be controlled with soil treatments of insecticide. However, insecticides such as chlordane and others are no longer registered for this use. Oftanol is presently registered but is not as effective as the materials that have been used in the past. During the past few years other alternative control techniques have been used. Some are aircraft cabin treatments with d-Phenothrin, trap crops, soil liming and pheromone and bait traps.

Laboratory and field studies were performed in an attempt to improve old techniques and develop new ones that may be useful to control Japanese beetles at airports in the future.

When trapping beetles at heavily infested airports, a major problem is having the manpower to empty all traps before they become overflowing and no longer effective. Recently, at Dulles International Airport, Ron Addington developed a sock trap that was reported to be superior. A sock with the end cut out and treated with 5% Sevin dust was attached to a standard trap. As the beetles fell through, they contacted the material with mortality resulting. Beetles would pass through the trap and, therefore, there would be no manpower needed to empty traps. Socks would be dusted with Sevin on a weekly basis or more frequently if rain occurred.

A laboratory test was conducted with native beetles to test the residual effects of 8 insecticides. The materials were sprayed onto plastic petri dish bottoms and native beetles were collected and placed onto the treated surfaces for a period of time. Five beetles were exposed to each dish and mortality readings were made after 24 hours. Beetles were collected and exposed over a period of days.

Table 1. Percent mortality of Japanese beetle adults after a 24 hour exposure to treated petri dish surfaces.

Material		Days after treatment						
		1	3	6	8	13	16	21
d-limonine	10%	0	0	0	0	0	0	
d-Phenothrin	10%	100	100	100	100	100	100	40
Orthene	10%	100	100	100	100	100	100	100
Pounce	10%	100	100	100	100	80	50	60
Pydrin	10%	100	100	100	100	100	67	100
Pyrenone	6%	100	100	100	100	100	100	100
SBP-1382	10%	100	100	80	40	20	33	40
Sevin XLR	10%	100	100	100	100	100	100	100
Check		0	0	0	0	0	0	0

Adults were also collected and exposed to treated surfaces for a 5 second period and then removed and held in untreated dishes for 24 hours.

Table 2. Percent mortality of Japanese beetle adults 24 hours after a 5 second exposure to treated petri dishes.

Material		Days after treatment				
		6	8	13	16	21
d-limonine	10%	0	0	0	0	0
d-Phenothrin	10%	100	20	40	50	60
Orthene	10%	100	100	0	50	100
Pounce	10%	100	40	40	0	60
Pydrin	10%	100	100	100	100	100
Pyrenone	6%	100	100	100	100	100
SBP-1382	10%	100	0	0	0	20
Sevin XLR	10%	67	100	100	83	100
Check		0	0	0	0	0

Pydrin gave the quickest kill of all materials tested. For the treatment of sock traps, Orthene, Sevin, Pyrenone and Pydrin should be tested. Pydrin gave the quickest kill and appeared to be the most effective of the materials tested.

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